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Samuel James Gradle
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GAUGING THE IMPORTANCE OF MICROHABITAT IN QUALITATIVE

MACROINVERTEBRATE SAMPLING IN AN EFFLUENT DOMINATED STREAM

(TITLE)

BY

SAMUEL JAMES GRADLE

THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

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IN THE GRADUATE SCHOOL, EASTERN ILLINOIS UNIVERSITY
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GAUGING THE IMPORTANCE OF MICROHABITAT IN QUALITATIVE MACROINVERTEBRATE SAMPLING IN AN EFFLUENT DOMINATED STREAM

By

Samuel J. Gradle

B.S. Southern Illinois University Edwardsville, 2014

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ABSTRACT

In the past different sampling strategies have been used to relate macroinvertebrate assemblages with habitat quality in the Sangamon River, above and below the sanitary district effluent discharge in Decatur, IL. The standard 20 jab method of proportional sampling in multiple microhabitats, based on QHEI physical habitat score, sampled allowed for comparison between sites based on overall community composition. However, it oversampled fine sediments, which dominate the Sangamon, therefore potentially missing sensitive taxa in isolated quality habitats. In the fall of 2016 I tested an enhanced qualitative approach to better gauge the importance of microhabitat types to macroinvertebrate assemblages in the river. We sampled five different natural microhabitats (riffles, fine sediments, root wads, snags, leaf packs) and 2 artificial substrates (Hester Dendy samplers, artificial leaf packs) at seven different sites. Sampling a subset of specific microhabitats allow for comparisons between sites, capture of sensitive taxa, and identification of specific habitats important in reclamation efforts. Non-metric multidimensional scaling (NMDS) in conjunction with a PERMANOVA and two-factorial MANOVA tests showed there were significant differences in assemblages between microhabitat types and between upstream and downstream sampling sites. Results indicate that root wad microhabitats are distinct from other microhabitat's assemblage structure because they harbor more sensitive taxa than any other microhabitat thus making it an ideal habitat to sample in this system. However, microhabitat assemblage structure was found to be heavily influenced by physical factors (QHEI and flow) overshadowing any potential effects of water quality alteration provided by the effluent. Ultimately, changing the flow patterns of the Sangamon to replicate a more

temporal-based/natural regime, rather than the current altered one, would in turn minimize variation in physical factors between sampling sites.

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INTRODUCTION

Aquatic macroinvertebrates are routinely sampled as part of habitat assessment of lotic systems. They are useful as bioindicators of water quality, flow, and physical habitat (Cortes et al., 2002; Dewson et al., 2007; Hooda et al., 2000; Oliveira & Callisto, 2010) and allow for comparisons between comparable sized waters in the same geographic area (Davies & Jackson, 2006; Yoder & Rankin, 1996). Aquatic macroinvertebrates comprise a diverse group of organisms, including annelids, mollusks, and arthropods, of which immature insects predominate. They can be found in virtually all aquatic ecosystems and are a critical component in both lentic and lotic systems. In flowing systems, macroinvertebrates can be found in a variety of microhabitats ranging from the headwaters to the mouth. Taxa are often categorized into functional guilds (i.e. shredders, grazers / scrapers, collectors, predators) based on their role in the trophic web. The river continuum concept (Vannote et al., 1980) predicts that the proportion of the various guilds will change with stream order, based on nutrient availability. Shredders process large organic material and should dominate first order streams; or areas with submerged leaves/organic debris (Dudgeon & Wu, 1999; Janke & Trivinho-Strixino, 2007; Wallace & Whiles, 1997). Grazers/scrapers feed on attached primary producers, provided by higher light levels of middle order stream. Collectors feed on small organic materials that tend to accumulate in high orders rivers. Predators should be present throughout. Macroinvertebrates are also a major food source for many fish and other aquatic vertebrates (Wallace & Webster, 1996).

Lotic macroinvertebrates also sort based on availability of specific microhabitats and oxygen availability. Flow rate and substrate coarseness are often complimentary

factors that can heavily influence the presence, density, and composition of macroinvertebrate communities. Coarse substrate (cobble and boulders), associated with higher flow in riffles and headwaters, provide refuge in areas with higher concentrations of dissolved oxygen required by some taxa (Cobb et al., 1992; Growns & Davis, 1994). Other taxa are tolerant of lower oxygen concentrations as seen in finer sediments in slow flow more characteristic of higher order reaches (Bertrab et al., 2013). Habitat complexity, both organic and inorganic substrates, is important for macroinvertebrates (Schmude et al., 1998). It influences food availability (ex. root wads collecting debris) and provides space for macroinvertebrates to dwell and hide, thus reducing predation (Diehl, 1992; Minshall, 1984; Rhodes & Hubert, 1991). Food availability alone can influence the presence of some taxa; for example submerged leaves/organic debris and presence of shredders (Dudgeon & Wu, 1999; Janke & Trivinho-Strixino, 2007; Wallace & Whiles, 1997).

Fish, macroinvertebrates, and algae have been used in biomonitoring in lotic systems because their assemblages generally reflect the quality of their aquatic environment. Fish, are longer-lived and motile, so they can reflect longer-term effects over larger areas. They also occupy multiple trophic levels, so community data like the Index of Biotic Integrity (IBI) can reflect impacts throughout food webs. However, sampling can be time consuming and costly (Fausch et al., 1990). In addition, metrics like IBI can be altered by other factors aside from degradation making them insensitive to small samples (Fausch et al., 1990). Benthic algae are sessile and their lifespan is relatively short. As such, they reflect localized impacts, but may not be as useful for detecting chronic effects. They also only represent one trophic level, primary producers.

Macroinvertebrates generally are numerous and easy to acquire, and many are relatively sedentary (though are vulnerable to drift) throughout their life in water. Their life histories range from multivoltine (multiple generations per year) to semivoltine (less than one generation per year), and they represent multiple trophic levels (Williams & Feltmate, 1992). Macroinvertebrates also have a large range of tolerances to pollution and other environmental factors (Barbour et al., 1998; Merritt, Cummins, & Berg, 2008). Because of these characteristics, macroinvertebrates have been used to examine the effects of dams on lotic processes (Ogbeibu & Oribhabor, 2002) and wastewater effluent on stream water quality (Baa-Poku et al., 2013; Canobbio et al., 2009; Nedeau et al., 2003).

Many different population indices are used when assessing macroinvertebrate assemblages. These include standard ecological measures like diversity, evenness, and taxonomic richness. Indices representing specific macroinvertebrate groups based on overall tolerance are also common. Examples include percent EPT (Ephemeroptera, Plecoptera, Trichoptera), Chironomidae, mayflies, and intolerant taxa (tolerance value of ≤ 3) using Hilsenhoff values (Hilsenhoff, 1982, 1987). Taxa of different habitats and trophic levels are used to evaluate impact of physical factors like habitat heterogeneity and food availability. Trophic-driven indices include percent scrapers, collectors, shredders, predators, and filterers. Habit-relevant indices include percent sprawlers, clingers, climbers, swimmers, and burrowers. Biotic metrics such as MBI (macroinvertebrate biotic index) are used to represent overall assemblage quality (Hilsenhoff, 1982, 1987). MBI incorporates the abundance and tolerance values of all taxa collected and gives an average score representing the tolerance score of the entire

group. Higher MBI numeric scores reflect more tolerant taxa and indicate a degraded habitat. Lower MBI scores reflect more intolerant taxa and indicate a higher-quality habitat.

The availability and types of microhabitats present are major factors in macroinvertebrate community structure. By providing variations in food and oxygen levels, habitat heterogeneity will harbor dissimilar assemblages (Costa & Melo, 2008; Sudduth & Meyer, 2006; Wood & Sites, 2002) thus may increase temporal stability (Brown, 2003, 2007) and community resilience to disturbances (Hax & Golladay, 1998; Negishi et al., 2002; Rasmussen et al., 2012). Consequently collection strategies that sample different habitat types provide different results (Lenz & Miller, 1996). Despite this, physical habitat scores are often only loosely correlated to macroinvertebrate assemblage trends and population indices. For example, the Qualitative Habitat Evaluation Index (QHEI) generates a score from 0-100, 0 being the lowest score for habitat quality and 100 being the highest, based on the physical factors present at the site. However, this method is primarily based on habitats for fish and not macroinvertebrates (D'Ambrosio et al., 2014; Rankin, 1989; Sullivan et al., 2004), so better QHEI score will not always predict higher quality macroinvertebrate assemblages.

Two macroinvertebrate-sampling methods are commonly used in Illinois. The first is the multi-habitat dipnet procedure (20-jab) (Barbour et al., 1998) which proportionally distributes 20 sub-samples (jabs) to multiple habitats based on their percent cover of the sampling site. Percent cover of habitats/substrate is typically determined using QHEI (combined 20 jab QHEI). The second is the Illinois RiverWatch method. This procedure collects from the two most "high quality" microhabitats: riffles,

leaf packs, roots wads/snags, undercut banks, and fine sediments, ranked from highest to lowest quality (*Illinois RiverWatch Stream Monitoring Manual*, 2008). This procedure also uses a set list of 37 generalized taxa instead of the genus/species level identification used in the 20 jab QHEI. These methods can produce different results despite their regional overlap (Petry, 2015).

The 20 jab QHEI is a standardized, quantitative procedure that determines the overall macroinvertebrate community composition and can be used to compare different streams, and it is probably the widest used method in the Midwest (Bartosova, 2008; Hinz Jr. & Metzke, 2013; Maia & Fatava, 2016).The nature of the 20 jabs, however, may make it biased in streams heavily dominated by poor habitat (ex. sediments) and thus underestimate taxonomic richness. In addition, 20 jab was developed for wadable streams (Barbour et al., 1998), making it less applicable for moderate to larger sized rivers. Rivers of these sizes are typically fine sediment-dominated and may harbor the majority of their benthic diversity and productivity in marginal habitats like leaf and wood debris, which can be easily under-sampled with 20 jab sample (Benke et al., 1984; Neuswanger et al., 1982). Furthermore, Álvarez-cabria et al., (2011) reported that taxonomic composition can change under altered water quality conditions in some microhabitat types while others within the same system can be unaffected. Because of this, detecting changes in water quality using pooled samples collected from different habitat types can be difficult. Therefore, targeting specific microhabitats for each site may be more useful to quantify total richness.

RiverWatch, which was also developed for wadable streams, directly targets microhabitats usually preferred by sensitive/rare taxa that can be overlooked by 20 jab.

For example leaf litter, which is not targeted directly by 20 jab sampling procedures, can harbor unique macroinvertebrate communities (Kominoski & Pringle, 2009; Rubbo & Kiesecker, 2004; Stone & Wallace, 1998). However, RiverWatch's qualitative nature tends to overemphasize microhabitats that are generally considered "high quality" but overlook others that may contain important EPT or other taxa (Rhodes & Hubert, 1991; Roy et al., 2003; Wood & Sites, 2002). A system that lacks high quality habitats, may have most of its local taxa reside in the more traditional "lower quality" microhabitats. This overestimation of quality is common with volunteer-based sampling methods (Engel & Voshell, 2002; Petry, 2015). Additionally, comparing different streams is difficult using RiverWatch.

The Sangamon River, a 246 mile (396 kilometers) tributary of the Illinois river (USGS, 2017a), is an example of a mid-sized river dominated by fine sediments. It is impounded at Decatur, Illinois, and areas immediately downstream of the impoundment have low habitat diversity and QHEI scores due to channelization, and altered unsteady flow, as regulated by the city of Decatur (Horsak et al., 2009; Lau et al., 2017; Shen & Diplas, 2010). Municipal effluent from the Sanitary District of Decatur enters the river approximately two miles downstream of the dam. Reaches farther downstream of the dam have higher QHEI scores and habitat diversity, due to increased and consistent flow provided by the effluent discharge (Nedeau et al., 2003).

Beginning in 2002, the Sanitary District of Decatur has collaborated with Eastern Illinois University to assess effects of municipal effluent on the downstream biotic communities. Both 20-jab and RiverWatch methods have been utilized to sample macroinvertebrates. Due to low QHEI scores (low habitat heterogeneity), most jabs from

sites near the impoundment were collected from fine sediments rather than more productive habitat types. Because the downstream sites have more higher quality habitats due to increased flow, a higher proportion of jabs were done in higher quality habitats. This uneven sampling made it difficult to determine whether assemblage differences were because of effluent or flow. To address this, a modified version of RiverWatch sampling, in which the four highest quality habitats are sampled instead of two, was used. This collected more sensitive taxa at the upstream sites than 20 jab (Colombo et al., 2014; Colombo et al., 2015). However, the imbalance of habitat heterogeneity between upstream and downstream sites was still an issue, because upstream sites lack riffle habitats which are always sampled if present. Using a method that controls for habitat would be better for direct comparison of flow-impacted upstream versus effluent-impacted downstream sites in the Sangamon.

In this study, I sampled macroinvertebrates in 2016, using a combination of 20 jab QHEI and RiverWatch methods, with 3 goals. The first objective was to assess the importance of specific microhabitats to macroinvertebrates in the Sangamon River. The second objective was to compare these habitat-specific samples to a composite 20 jab sample based on QHEI to assess potential sampling bias. The third objective was to assess any effects of the effluent on the downstream communities by comparing macroinvertebrates collected from equivalent microhabitats. The goal of this study is to reveal which microhabitats are the most important for resident macroinvertebrates in this heavily impacted system and, therefore, which should be targeted in any future efforts at remediation/habitat improvement in the Sangamon River.

METHODS

Study Sites

Macroinvertebrate assemblages were sampled from seven sites in the Sangamon River near Decatur Illinois during 2016. The sample reach extended from just below the Lake Decatur dam in Decatur to roughly 20 river miles downstream, past the Sanitary District of Decatur, towards Springfield Illinois (Figure 1). Three sites were located upstream of the effluent discharge of Decatur's sanitary district and four sites were located below the effluent discharge. Each site was approximately 100 meters in length.

Sampling

On October 3rd, 5th, and 6th of 2016, five different microhabitat types (riffles, leaf packs, root wads, snags and fine sediments) were sampled at each site. Three replicates (subsamples) were collected from each microhabitat type present at the site, each subsample or "jab" was taken with an 18 inch square frame dipnet with a 500 μm mesh. Jabbing procedures varied by microhabitat type and were done according to methods described in the EPA macroinvertebrate multihabitat sampling protocol and RiverWatch methods (Barbour, et al. , 1998; *Illinois RiverWatch Stream Monitoring Manual*, 2008). The contents from each jab were concentrated using a bucket sieve, individually placed in a sampling jar, and preserved with 95% ethanol. Samples were labeled with the site number, microhabitat type, and any unique details about the sampled habitat (ex. if fine sediments were sand or silt consistency).

Four artificial samplers were placed at each site four weeks prior to microhabitat sampling. These samplers consisted of a fourteen plate Hester-Dendy sampler and a mesh

bag (polypropylene onion sack) filled with 24 grams of dried leaves (an equal proportion of silver maple, cottonwood, and sycamore leaves) zip-tied to a brick, and anchored at each site in areas beneath (slightly under) partially submerged root wads. During collection, three of these four samplers were selected at random from each site. Leaf bags were emptied into a bucket sieve, concentrated invertebrates were transferred into a jar and preserved with 95% ethanol. Hester-Dendy samplers were placed intact into wide mouth jars and preserved with 95% ethanol as well. All samples were taken back to EIU for processing and identification.

Habitat Evaluation

Physical habitat at each site was assessed using the Qualitative Habitat Evaluation Index (QHEI) (Rankin, 1989). Depth and substrate type were recorded in two feet increments from three equidistant (50 meters apart) transects which were measured along the width of the river. The current velocity was also recorded, at three equally spaced points along the transect, to estimate the flow and determine a profile.

Processing and Identification

Samples from each habitat were subsampled, using a thirty-grid subsampling tray. A minimum of three random grids (10% of total sample) and at least 200 individuals were picked (King & Richardson, 2002; Oliveira, et al. , 2011). Large and/or rare taxa were picked after subsampling and were recorded as being picked afterward to accurately calculate estimated abundance for these taxa. Individuals were identified to genus if possible; except for members of the family Chironomidae which were identified down to subfamily and tribe (Merritt, et al. , 2008).

Data Analysis

Differences in QHEI scores (including derived sub scores such as channel morphology, substrate quality, pool/riffle quality, instream cover, riffle quality, amount of bank erosion) and estimated flow between the two reaches were tested using Student's T-tests. Estimated abundance for macroinvertebrate data was calculated for each sample and were then converted to relative abundance data to account for differences in estimated abundance between microhabitats possibly caused by differences in sampling strategy.

Non-metric multidimensional scaling (NMDS) was used to produce a visual representation of the similarity between assemblage data with the vegan package in Rstudio (Oksanen et al., 2017). Assemblage data is presented by between site locations (upstream of effluent (sites 3, 5, & 7), immediately downstream of effluent (9 & 12), and far downstream of effluent (14 & 15)) and microhabitat types. Specific driving factors, including physical data collected from QHEI, flow measurements and important taxa (Oksanen et al., 2017) were incorporated in the site location plot using the *envfit* function in the vegan package. Separate plots were also made for each microhabitat type to look for difference between sites. Important taxa were also incorporated into these plots. PERMANOVA with 999 permutations was done for each plot type with the use of the *adonis* function in the vegan package to compare assemblage similarity based on site and habitat type. Data was square-root transformed to fulfill assumptions of normal distribution and homogeneity of variance.

General population indices including MBI, Simpson's index of diversity, richness, percent EPT, percent intolerant (tolerance value ≤ 3), and percent mayflies were

calculated. In addition, a unique EPT based value percent non Hydropsychidae and Polycentropodidae EPT was calculated for this study. In order to exclude common moderately tolerant Hydropsychidae and Polycentropodidae caddisflies from more sensitive/rare taxa EPT taxa. It is common to exclude Hydropsychidae taxa from EPT indices for this reason (Boehme et al., 2016). MBI and Simpsons index of diversity were calculated using the following formulas.

$$MBI = \frac{\sum T_i N_i}{N}$$

[T_i = tolerance value of each taxa, N_i = number of individuals of each taxa, N = total abundance].

$$Simpson's\ Index\ of\ Diversity = 1 - \frac{\sum n(n-1)}{N(N-1)}$$

[n = total number of individuals of a species, N = the total number of individuals of all species]

Other, more trophic or habit based, indices were calculated as well to account for more physical/food availability factors. These included percent collectors, scrapers, shredder, clingers, sprawlers, and swimmers. Separate two-factorial MANOVAs were used to detect differences in microhabitat type and site as assessed by general versus trophic/habit based indices. The TukeyHSD was used for the post hoc tests for univariate results and data was double square-root transformed.

A composite 20 jab sample was calculated for each site from a proportional representation of individual microhabitats, as determined by QHEI. For example, if 15% of the sampling site area were root wads, then the data from three root wad samples (one

jab for every 5% makeup of that habitat type) from that site would be used in the 20 jab sample. Replicates for each microhabitat were combined to calculate indices (MBI, percent EPT, non Hydropsychidae/Polycentropodidae EPT, Simpson's diversity, richness, scrapers, shredders, clingers, collectors, sprawlers, swimmers, mayflies, and intolerant taxa) separated by site and were also double square root transformed and compared to 20 jab samples using post hoc results to a series of one factorial ANOVAs.

RESULTS

Biological Survey

A total of 26,574 individuals were identified from 135 samples collected This included 59 genera from 42 families and 13 orders (Appendix 1). Samples were dominated by members of the families Chironomidae and Hydropsychidae (Appendix 2). Gastropods in the genera Fossaria, Menetus, and Pleurocera were unique to the upstream sites; while taxa such as mayflies of the families Baetidae, Caenidae, Isonychiidae, Leptohyphidae, Corydalidae, Hydroptilidae, Philopotamidae, Aeshnidae, Calopterygidae, Leptohyphidae, including the genera Heptagenia and Polycentropus were unique to the downstream sites. Other insect families such as Heptageniidae, Leptoceridae, and Elmidae were more common in downstream sites.

Comparison of Microhabitat Types

Assemblages differed between microhabitat types (PERMANOVA results p = 0.001). Riffle assemblages were driven by mayflies, Megaloptera and riffle beetles (Figure 2). Rootwads were dominated by Odonates. Artificial leaf packs and Hester Dendy samples were driven by snails, amphipods, and one Trichopteran genus (Cyrnellus). Members of Chironomidae did not have a strong preference for any microhabitat type (Figure 2). Overall richness was 24 – 28 taxa in all microhabitat types, except root wads which had 40 taxa. Root wads had ten unique taxa (6 Odonata, 2 Elmidae, 1 Diptera and 1 Amphipoda) relative to all other microhabitats, and riffles had two unique taxa (1 Elmidae, and 1 Trichoptera) not found in root wads.

Artificial leaf packs generally had lower population indices values than other microhabitats. When these samplers were collected, however, they were partially embedded in sediment which may have altered their assemblage structure. Artificial leaf packs were significantly different from natural leaf packs, as measured by non-Hydropsychidae/Polycentropodidae EPT ($p = 0.023$) and percent swimmers ($p = <0.001$).

Root wads differed from other microhabitats, on average, using six of the 13 metrics, whereas the other microhabitats varied by approximately four (Appendix 3). Root wads were significantly higher than other microhabitats in percent makeup in non Hydropsychidae/Polycentropodidae EPT, shredders, and intolerant taxa than any other microhabitat type (Figure 3, Appendix 3). It should be noted, however, the only shredder taxa collected, in large enough numbers to have any impact on analyses, was the trichopteran genus Nectopsyche. Root wads were significantly lower in collector and clinger percent makeup than any other microhabitat type (excluding artificial leaf packs) (Figure 3, Appendix 3). Natural leaf packs were significantly higher in percent shredder makeup that any other natural microhabitat type except for root wads (Figure 3, Appendix 3). Snags, riffles, and leaf packs were significantly higher in percent makeup of swimmer taxa than all other microhabitat types except for root wads (Figure 3, Appendix 3).

Microhabitats and 20 Jab QHEI

Composite 20 jab samples were significantly different from individual microhabitats with five indices (percent shredders, scapers, sprawlers, and swimmers) (Figure 3, Table 1). Composite 20 jab samples were also significantly higher in richness than Hester Dendy samplers, sediments, and snags. Most proportional indices (MBI,

percent EPT, non Hydropsychidae/Polycentropodidae EPT, clingers, collectors, mayflies, and intolerant taxa) were not significantly higher with 20 jab samples (Figure 3). However, percent shredders for Hester Dendy samplers, riffles, sediments, and snags were significantly different from 20 jab samples (Figure 3). Sprawler taxa for artificial leaf packs, Hester Dendy samplers, sediments, and snags were significantly different for 20 jab samples. Swimmer taxa were significantly different in root wads, sediments, and snags than in 20 jab. Scrappers were also significantly different with root wads than 20 jab.

Comparisons of Upstream and Downstream Sites

QHEI scores from the 7 sites ranged from poor (<45) to excellent (>77) quality (Figure 4). Scores were higher for the downstream sites, but not significantly different ($p = 0.23$) (Figure 4). There was, however, a significant ($p = 0.041$) increase of average flow in the downstream sites (Figure 4). The trend was that physical factors such as average flow and values derived from QHEI scores (level of erosion, substrate, channel morphology, and riffle quality) increased going from upstream to downstream sites.

Total macroinvertebrate assemblages grouped by upstream, immediate downstream, and far downstream sites, relative to effluent outfall differed significantly between sites (PERMANOVA $p = 0.001$) (Figure 5). Assemblages from individual microhabitat types also differed by site (Figure 6) with all $p < 0.01$. All microhabitat types showed a similar trend with most EPT taxa (Baetidae, Isonychiidae, Heptageniidae, Leptohyphidae, Hydropsychidae) and other intolerant and more riffle-specialized taxa being the driving forces for more downstream sites. Gastopoda, Oligochaeta and Chironomini taxa, in contrast, were more prevalent in most upstream sites.

Patterns were variable, but population indices showed an overall improving trend in MBI, percent EPT, non Hydropsychidae/Polycentropodidae percent EPT, richness, percent scrapers, clingers, sprawlers, swimmers, and mayflies for farther downstream sites for most habitat types (Figure 3). Whereas percent shredders and intolerant taxa are more dependent on microhabitat.

MANOVA analysis of general population indices (MBI, richness, Simpson's diversity, percent EPT, non Hydropsychidae/Polycentropodidae EPT, mayfly, intolerants) indicated an influence of both microhabitat and site with interaction (all $p < 0.05$) (Table 2). Trophic and habit indices yield similar results with a significant difference for both factors with interaction (all $p < 0.05$) (Table 3). After controlling for microhabitat type, univariate analysis showed that there were significant differences between sites (Figure 3). Most significant differences were seen when comparing the two most downstream sites (14 and 15) to the rest of the sites (Appendix 4).

DISCUSSION

Although artificial samplers are commonly used to sample macroinvertebrates because of their ease of use and low variability between samples (Arthur & Horning, 1969; Pauw et al., 1986), sampling natural habitats has been argued to be relevant as well (Casey & Kendall, 1997; Roby et al., 1978). One of the most commonly used methods is the 20 jab sample based on QHEI (Hinz Jr. & Metzke, 2013; Maia & Fatava, 2016). However, since it was developed for wadable streams, it may have limitations when used in larger rivers. This method of standardized quantitative sampling, based on proportional sampling of microhabitats present at a site, allows for straightforward comparisons. Though, it is time consuming and may overemphasize "poor" microhabitats in rivers dominated by fine sediments with patchy "quality" microhabitats. Thus, although useful for an overall assessment of a site, it may miss taxa found only in these lightly sampled microhabitats. It has been proposed that using a more qualitative technique targeting specific habitat types, is a viable alternative to more quantitative techniques for assessing water quality (Lenat, 1988). This study set out to compare different macroinvertebrate sampling strategies in a physically-altered river dominated by fine sediments.

In previous years, macroinvertebrate sampling in the Sangamon River showed an improvement in percent EPT and MBI scores as you proceed downstream from the dam of Lake Decatur (Colombo et al., 2014; Colombo et al., 2013; Colombo et al., 2015). However, these results were still notably different between years in which the 20 jab method was used compared to 2014 when a modified RiverWatch protocol was used. In these reports, though every year showed some assemblage based differences between

upstream and downstream sites of the effluent, only the 2014 sampling (RiverWatch protocol) detected a significant difference, as measured by percent EPT (Colombo et al., 2015). Microhabitat sampling in my study showed similar trends to the 2014 RiverWatch samples. Similarities between these sampling methods was expected because the same type of microhabitats were sampled at roughly equal proportions with both methods. In contrast, previous 20 jab samples did not detect differences between upstream and downstream sites based on assemblage composition.

Physical habitat scores, (QHEI) were consistently higher, although not significantly, in the down-stream sites than the upstream sites (Colombo et al., 2014, 2013). Even though physical habitat changed little between years in the Sangamon River, flow patterns were seemingly random. During the time when the 2016 sampling was conducted, gauge height for route 48 gauging station on the Sangamon never dropped below 2.5 feet and often climbed to six or eight feet, sometimes within 24 hours (USGS). Conversely, 2012, 2013, and 2015 sampling periods had consistently low flow (gauge height usually around two feet) during at least a one-month period in the late summer. A similar sampling strategy used in 2015 and this 2016 sampling showed different results which, perhaps due to the differences in flow patterns between years (Colombo et al., 2016). Whereas the flow patterns during the 2014 sampling were more similar to 2016 which may explain why results between these two years were more similar (Colombo et al., 2015).

The city of Decatur controls discharge over the Lake Decatur dam. Variable flow over the dam contributes to physical habitat impacts in sites between the dam and the

effluent outfall and effected water chemistry parameters. At low overflow, the river between the dam and effluent outfall is reduced to very low flow or disconnected pools and water chemistry values were much different between sites above versus below effluent outfall. However, high overflow maintained a flow regime with better available physical habitat and, at > 200 cfm overflow, water chemistry differences above versus below effluent outfall were marginal (Colombo et al., 2017). Earlier studies of this river (Ciak, 2007) suggested that the increase in habitat heterogeneity and QHEI scores in the farther downstream sites was due to the diminishing effect of the modified flow regime from the impoundment. This increase in availability of different microhabitats may help explain the increase in percent EPT and other indices with the farther downstream sites in previous sampling. This theory is supported in studies of other similarly altered systems (Voelz & Ward, 1989; Ward & Stanford, 1983) as well, and it is well documented that microhabitat type does influence community composition (Jowett et al., 1991; Orth & Maughan, 1983; Wood & Sites, 2002).

Microhabitats

Microhabitat type and site greatly influenced the assemblage composition and distribution of individual taxa. Artificial samplers (Hester Dendy multi-plate samplers and artificial leaf packs) were the most similar in assemblage composition (Figure 2), despite their structural difference and varied the least between sites. However, artificial samplers overall, and especially artificial leaf packs, had assemblages consisting of relatively tolerant invertebrates such as silk-cased caddisflies (genus: Cynellus) and Chironomidae larvae (Figure 6). This may simply be because Hester Dendy samplers and artificial leaf packs were attached to a common anchor located near root banks, which

can have high amounts of erosion. Artificial leaf packs were partially imbedded in sediments which may have altered their assemblage structure. Based on these findings, artificial samplers such as these may only be viable if deployed on courser substrates in this type of river system

Sediments, leaf packs and snags were also similar in assemblage composition (Figure 2), but leaf packs had significantly higher percent non Hydropsychidae/Polycentropodidae EPT and shredder taxa than sediments or snags. In addition, percent intolerant and swimmer taxa were significantly higher with leaf packs than sediments (Appendix 3). These differences were clearer with farther downstream sites (Figure 3). The higher number of shredders in leaf packs than the other two habitat types is to be expected based on available food. Increased percent swimmers and percent intolerant taxa may just be a reflection of leaf packs as the best available microhabitat at a site. Leaf packs were not found at site 3, just below the dam. This may be because of the combination of this system being channelized, flashy, moderate-sized river which reduced the retention of fallen leaves (Larranaga et al., 2003; Muotka & Laasonen, 2002), especially near the dam.

Riffles contained taxa more typical of courser substrate, including dobsonflies, torpedo mayflies, and clinging mayflies (Figure 2). Although they did contain a high amount of EPT and other sensitive taxa (Figure 3), riffles were also missing from many sites, limiting the potential habitat for these taxa in the Sangamon River.

Root wads also possessed a wide diversity of taxa, and, unlike leaf packs and riffles, root wads were present at each site. Relative abundance of shredders, intolerant taxa, non-Hydropsychidae/Polycentropodidae EPT taxa, and overall taxonomic richness

of root wads were significantly greater than any other habitat type collected (except richness for riffles). This coincides with other studies which have shown that submerged bank roots harbor unique taxa in streams (Wood & Sites, 2002) and root wads have been recommended to use for qualitative sampling over other habitat types (Álvarez-cabria et al., 2011). In this section of the Sangamon, root wads seem to be a productive habitat to a variety of sensitive taxa. This is likely because the structure of submerged roots provides more cover and substrate to harbor a diverse invertebrate community (Sudduth & Meyer, 2006). Furthermore, submerged bank roots can act as a sieve for drifting organic debris providing food for shredders (Rhodes & Hubert, 1991; Sudduth & Meyer, 2006), explaining their high abundance in root wads sampled (Figure 3). Root wad shredders all belonged to one genus (Nectopsyche) which are considered to be intolerant (tolerance value ≤ 3) according to the Illinois EPA (Lin, 2007). As such, they made up most of the intolerant taxa collected from the root wad samples. In other words, even though root wads possessed the most shredder and intolerant taxa of any microhabitat type, the pool of these two taxa groups was not very diverse. Although the shredder diversity in root wads was low, it was universally low with every microhabitat sampled that harbored any shredders. Additionally, because they harbored the most shredder taxa, root wads probably supplied most of the shredders to the overall community. This in turn can affect the composition of a composite sample like 20 jab which may under-sample root wads and instream cover in a fined substrate dominated system like the Sangamon.

Comparison to 20 jab QHEI

Individual microhabitat and 20 jab samples showed similar general trends, with most population indices increasing in sites farther downstream. However, there were

some differences between microhabitat and 20 jab samples. Higher richness in 20 jab than in microhabitat samples was expected since 20 jab samples are, by their nature, larger and include multiple microhabitat types. Other proportional indices (percent scrapers, sprawlers, shredders, and swimmers) were significantly higher in 20 jab samples. Although, root wads and leaf packs were the only two microhabitats that were not significantly lower in shredder taxa than 20-jab samples. Root wads were, in fact, higher (although not significantly) when compared to 20 jab samples, indicating that most shredder taxa came from root wad subsamples in the 20 jab composite samples. Thus, minor habitats like root wads in a larger river may be particularly important in harboring a variety of taxa not found in main channel habitats, and therefore contribute to a large portion of the overall macroinvertebrate diversity in the stream (Rhodes & Hubert, 1991). Leaf packs also did not differ significantly from the amount of shredder taxa from 20 jab. This is important to note because 20 jab samples do not directly collect leaf packs so any resident taxa would be missed in 20 jab samples. Thus, although they do not make up large percentage of the proportional 20 jab sample, as determined by QHEI dominated by fine sediments, root wads and leaf packs can contribute a large portion of sensitive and/or unique taxa.

These results indicate that the 20-jab sampling procedure may not be necessary for sampling this reach of the Sangamon River. If the objective is monitoring MBI, percent EPT, and other sensitive taxa based indices, both 20 jab and sampling multiple specific microhabitats would essentially detect a similar proportion of sensitive taxa. Even when considering indices that differ significantly between given microhabitats and

20 jab samples, simply sampling a few important microhabitat types at the same time, like the RiverWatch method, likely would be enough to remedy this inconsistency.

Effects of municipal effluent and physical characteristics

I expected to see notable changes to assemblage composition in sites closest to the effluent outfall point source. If there was an adverse effect of water quality, there should have been an immediate effect on the closest downstream site consistently for each microhabitat, but this was not the case. The trends shown with MBI and other population indices are inconsistent, but do not reflect the trend of a point source effect. In preparing NMDS plots, sites were originally sorted by upstream sites (3, 5 and 7), immediately downstream sites (9 and 12), and far downstream sites (14 and 15), based on their spatial relationship to effluent outfall. In fact, based on overall assemblage structure, site 7 (located immediately upstream of the effluent) was more like downstream sites 9 and 12 than it was to sites 3 and 5 (the other upstream sites) (Figure 4). This suggests that the effluent has less effect on the downstream macroinvertebrate communities than physical factors, dependent on flow (Figure 4).

Others have suggested that physical parameters such as habitat availability can have an equal or greater impact on resident macroinvertebrate communities than chemical factors (Nedeau et al., 2003, Arthur & Horning, 1969). Many population indices generally improved going farther downstream. This may have been a result of the impoundment, but it was not a consistent steady increasing trend when going farther downstream. Instead, changes look more abrupt and random. These fluctuations, however, did correlate loosely with QHEI scores and flow (Figure 5). Despite the notable differences in water quality (conductivity; concentrations of ammonia, phosphorous, and

chloride) between upstream and downstream sites during the time of the sampling (Colombo et al., 2017) there is no apparent influence of water quality from the effluent on macroinvertebrate assemblage, this is shown in Figure 5.

The polarizing differences of the two farthest downstream sites (14 and 15) and the two most upstream sites (3 and 5) provides further evidence that physical factors have a much more substantial effect on site composition. Assemblages at sites 3 and 5 consisted of more generalized/tolerant taxa typical of low habitat diversity. Sites 14 and 15, in contrast, harbored more sensitive taxa typically found in areas of course substrate as well as overall higher habitat diversity, such as swimmers and clingers (Figures 5 and 6). These differences coincided with physical parameters because sites 3 and 5 had some of the lowest flow and QHEI scores, whereas 14 and 15 had the highest flow and QHEI scores. Overall improving trends in farther downstream sites, despite having generally lower water quality, further supports the assumption that increasing flow and habitat quality effects assemblage composition more than a decrease water quality.

Patterns, relative to differences in indices between sites, varied when examining specific microhabitat types. Although root wads possessed the largest number of sensitive taxa, they also had high variability between sites. This was especially apparent in shredder, non-Hydropsychidae/Polycentropodidae, and intolerant taxa. There are very few of these taxa in root wads for upstream sites, and the patterns displayed do not follow QHEI or flow trends between sites as well as other indices. It is unclear what caused this, but one explanation could be the Sangamon's dramatic and inconsistent flow patterns. Since 2016 was an especially flashy year, this may have altered the macroinvertebrate community. The flashiness of the Sangamon was perhaps felt more abruptly at sites

closer to the dam and would make bank microhabitats like root wads more vulnerable to desiccation than other microhabitats. This would, in turn, greatly effect colonization of some macroinvertebrates because it would be easier for taxa with shorter generation times (ex. Chironomidae) to recolonize. Thus these taxa would have a more rapid recovery and be more persistent in these upstream microhabitats than taxa (ex. Ephemeroptera) with longer generation times (Molles, 1985). In contrast, downstream root wads receive a constant source of flow from the effluent discharge and therefore are less vulnerable to dramatic changes in water level.

Nedeau et al. (2003) suggested that the industrial effluent discharging into Portage Creek in southwest Michigan increased flow and consequently improved habitat heterogeneity. This is very similar to what seems to be happening with the Sangamon River. If this is the case, not only does the effluent help cushion the effects of flashiness directly, it also helps increase habitat heterogeneity, which can aid in recovery from spates or times or drought (Brown, 2007; Negishi et al., 2002) by making root wad microhabitats more resilient in downstream sites. If there was a more consistent flow pattern, root wads, and possibly other microhabitat types, closer to the dam should likely harbor a larger diversity of more environmentally sensitive taxa and thus be more comparable to the root wads located farther downstream.

CONCLUSIONS

In summary, the type and number of microhabitats sampled are important factors when examining the macroinvertebrate community of a river such as the Sangamon. Sampling methods like those used in this study may not necessarily produce better results than 20 jab, but they yield similar patterns for many indices. This suggests that sampling a few quality microhabitats may be a quicker alternative to 20 jab when examining water quality in a physically-altered stream such as the Sangamon. For future sampling endeavors for this river and other rivers like it, I would suggest continuing to utilize the modified version of RiverWatch used in previous years while keeping microhabitats as separate samples and not pooling them into one composite sample. Special attention should be directed to root wads because they can contain many sensitive taxa and are present throughout the river. In addition to this, I would suggest utilizing some artificial samplers. Artificial leaf packs should be used again but must be suspended in the water away from sediment to prevent them from getting buried. Using a sampler to mimic root wads should be considered because sensitive taxa was found in root wad samples. Furthermore, using a standardized artificial version of root wads would reduce variability. The main issue when it comes to macroinvertebrate communities in the Sangamon, however, is the altered flow from the dam. The Sangamon River is atypical in that the addition of sanitary effluent may improve conditions in the river. Nutrient loads are certainly greatly increased, but intermittent flow over the dam negatively impacts physical habitat and thus macroinvertebrates. Consistent flow supplied by the effluent outfall is arguably the critical component in a lotic system. A consistent flow regime

would assure habitat persistence, allow more sensitive/rare taxa to colonize near the dam, and should be the first step in any remediation work on the Sangamon River.

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Table 1. Adjusted p-values from the post hoc results of the one-way ANOVAs comparing the different habitat types (through population indices) to 20 jab samples. Richness, scraper, shredder, sprawler, and swimmer taxa with some of the habitats showed to be significantly different from 20 jab samples.

Indices Type	Art. Leaf Pack	Hester Dendy	Leaf Pack	Riffle	Root Wad	Sediments	Snag
MBI	0.998	0.999	0.725	0.220	0.841	0.982	0.999
Richness	0.064	0.027	0.856	0.606	0.324	0.005	0.026
Simpson's Diversity	0.846	0.955	1	0.938	0.997	0.962	0.999
Pct. EPT	0.980	0.997	1	0.814	1	1	0.920
Pct. Non-hyd./poly. EPT	0.398	0.994	1	0.959	1	1	0.999
Pct. Scraper	1	0.981	0.988	0.998	0.007	0.978	0.788
Pct. Shredder	0.113	0.024	0.999	0.033	0.081	0.019	0.022
Pct. Collector	0.873	0.991	1	0.991	0.101	1	1
Pct. Clinger	0.604	1	1	0.876	0.692	1	0.852
Pct. Sprawler	0.036	<0.001	0.062	0.761	0.672	0.001	0.002
Pct. Swimmer	0.078	0.247	0.042	0.578	0.003	0.001	<0.001
Pct. Mayfly	0.999	0.991	1	0.997	1	0.922	0.979
Pct. Intolerant	0.791	0.677	1	0.999	0.935	0.882	0.842

Table 1. Summary table of the two-factorial MANOVA statistical results evaluating the generalized population indices using Pillai's trace. Displaying degrees of freedom, mean squares, F statistics, and p-values.

	df	MS/Pillai's Trace	F	P
Multivariate test				
Site	1, 115	0.6757	34.236	<0.0001
Habitat	6, 720	1.5564	6.004	<0.0001
H x S	6, 720	0.6999	2.264	<0.0001
Univariate tests				
MBI				
Site	1	0.1348	80.58	<0.0001
Habitat	6	0.0122	7.3	<0.0001
H x S	6	0.0028	1.7	0.1265
error	121	0.0017		
Percent EPT				
Site	1	20.179	68.12	<0.0001
Habitat	6	1.8898	6.38	<0.0001
H x S	6	1.6435	5.55	<0.0001
error	121	0.2962		
Simpson's Diversity				
Site	1	0.0248	6.12	0.0147
Habitat	6	0.0234	5.78	<0.0001
H x S	6	0.0089	2.2	0.0472
error	121	0.0041		
Richness				
Site	1	0.656	36.79	<0.0001
Habitat	6	0.1564	8.77	<0.0001
H x S	6	0.0275	1.54	0.1695
error	121	0.0178		
Non-Hyd./Poly. percent EPT				
Site	1	44.379	147.85	<0.0001
Habitat	6	3.998	13.32	<0.0001
H x S	6	0.982	3.27	0.0051
error	121	0.3		

Percent mayfly				
Site	1	40.161	130.61	<0.0001
Habitat	6	1.431	4.66	0.0003
H x S	6	1.014	3.3	0.0049
error	121	0.307		
Percent intolerant				
Site	1	9.7366	49.43	<0.0001
Habitat	6	6.459	32.79	<0.0001
H x S	6	0.4082	2.07	0.0614
error	121	0.197		

Table 2. Summary table of the two-factorial MANOVA statistical results evaluating the habit and trophic population indices using Pillai's trace. Displaying degrees of freedom, mean squares, F statistics, and p-values.

	df	MS/Pillai's Trace	F	P
Multivariate test				
Site	1, 116	0.7422	55.663	<0.0001
Habitat	6, 726	1.4025	6.154	<0.0001
H x S	6, 726	0.988	3.975	<0.0001
Univariate tests				
Percent scraper				
Site	1	8.1599	21.01	<0.0001
Habitat	6	2.2432	5.78	<0.0001
H x S	6	1.1078	2.85	0.0124
error	121	0.3884		
Percent shredder				
Site	1	4.8134	31.47	<0.0001
Habitat	6	5.4727	35.77	<0.0001
H x S	6	0.9073	5.93	<0.0001
error	121	0.153		
Percent clinger				
Site	1	7.1342	52.54	<0.0001
Habitat	6	1.2472	9.19	<0.0001
H x S	6	0.1283	0.95	0.4656
error	121	0.1358		
Percent collector				
Site	1	0.154	7.74	0.0063
Habitat	6	0.213	10.7	<0.0001
H x S	6	0.0766	3.85	0.0015
error	121	0.0199		
Percent sprawler				
Site	1	6.8027	27.16	<0.0001
Habitat	6	1.1531	4.6	0.0003
H x S	6	0.5291	2.11	0.0567
error	121	0.2505		

Percent swimmer				
Site	1	17.445	95.37	<0.0001
Habitat	6	2.3352	12.77	<0.0001
H x S	6	1.594	8.71	<0.0001
error	121	0.1829		

FIGURES

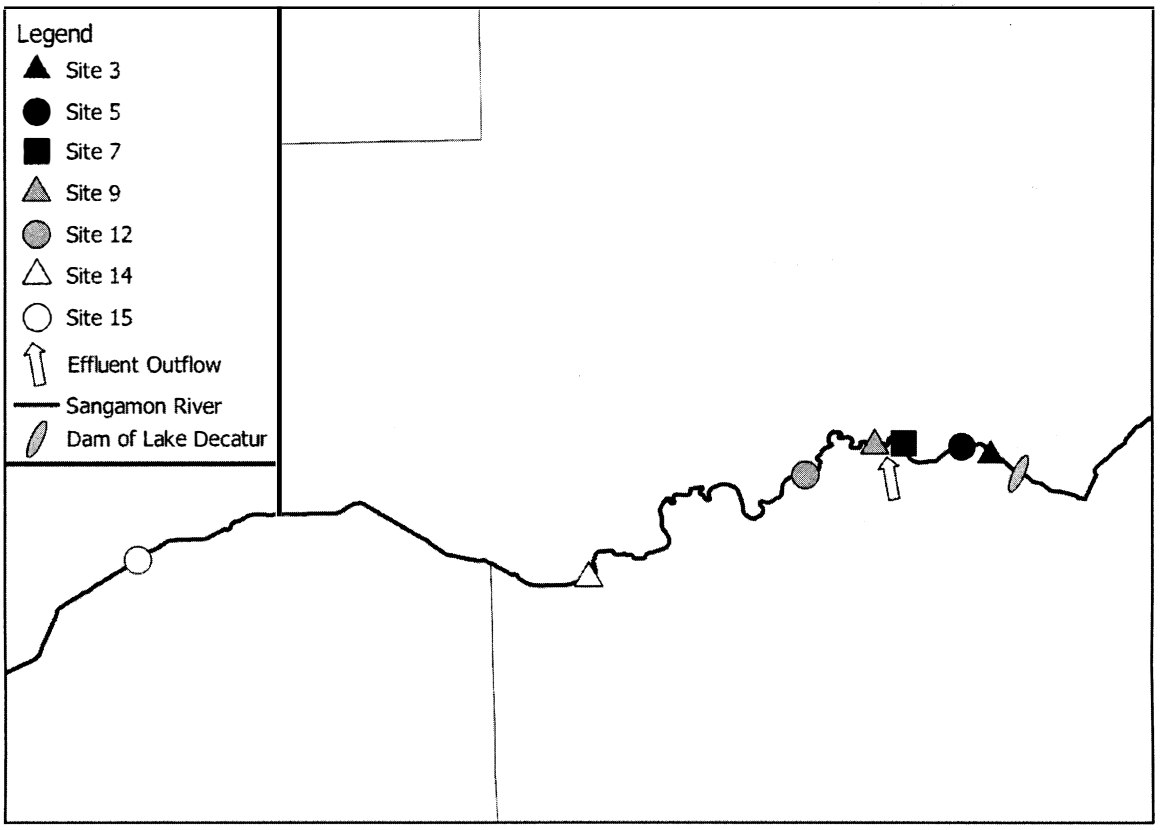


Figure 1. Sites sampled along the Sangamon River in fall of 2016.

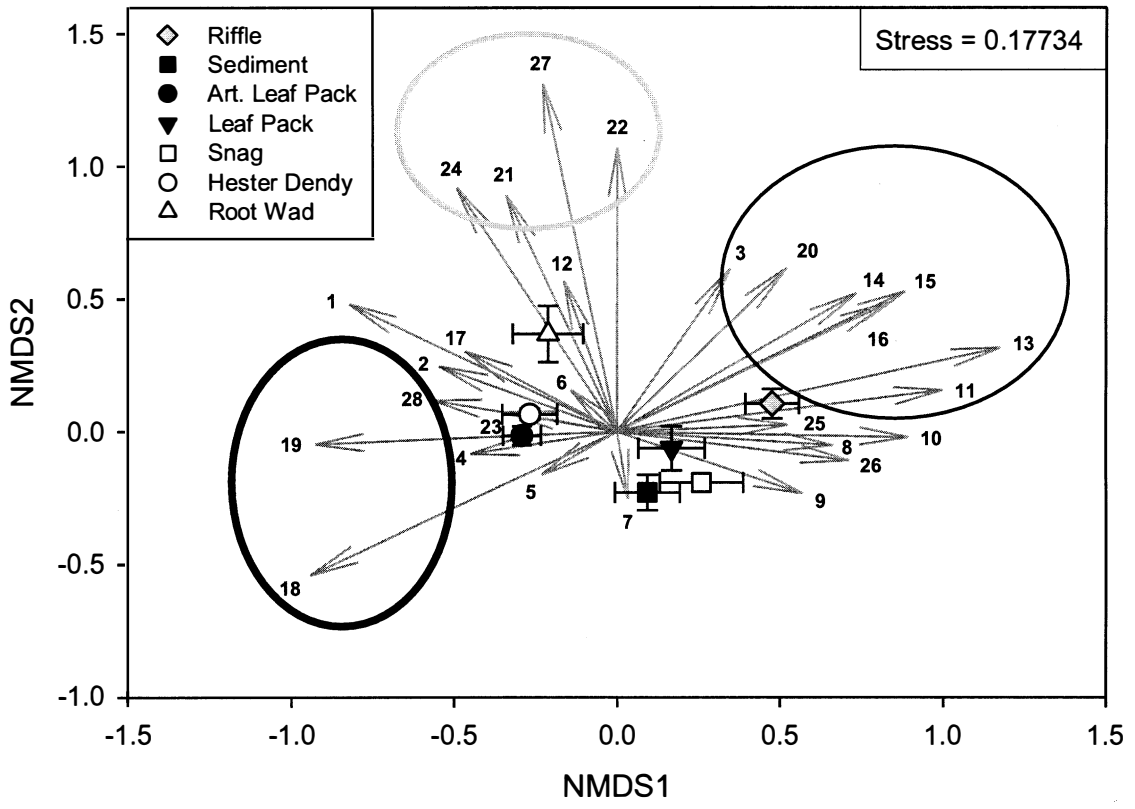
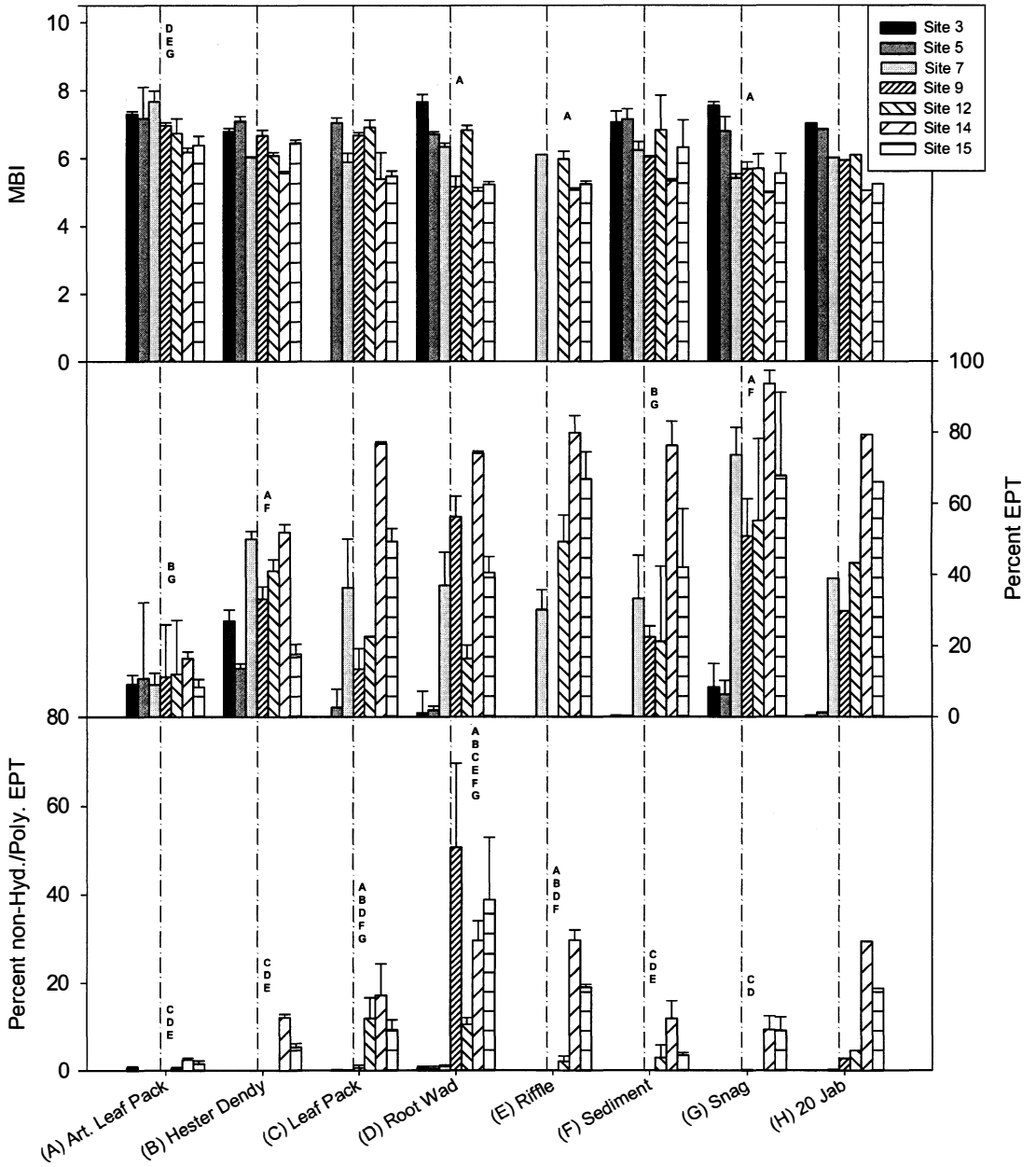
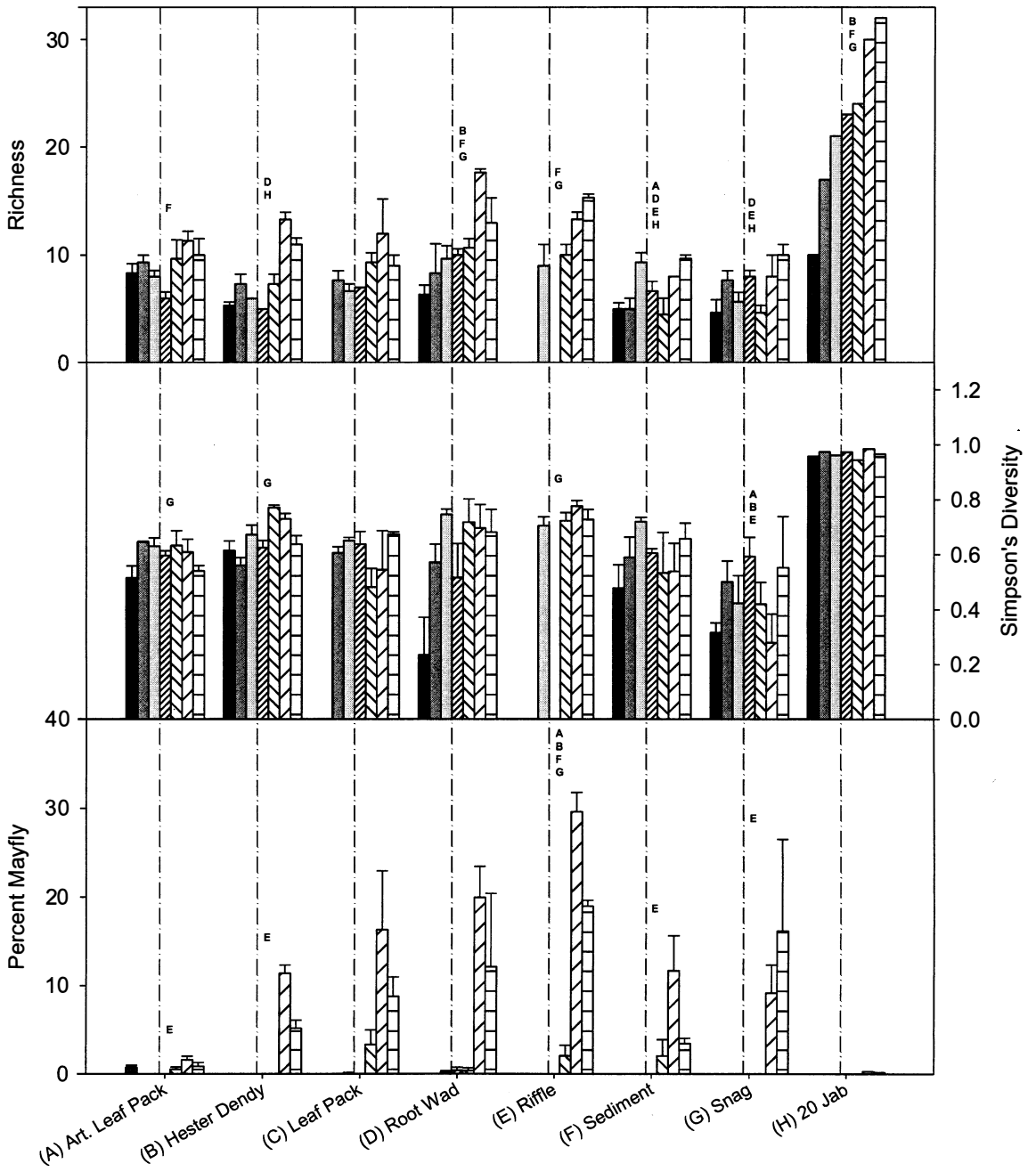
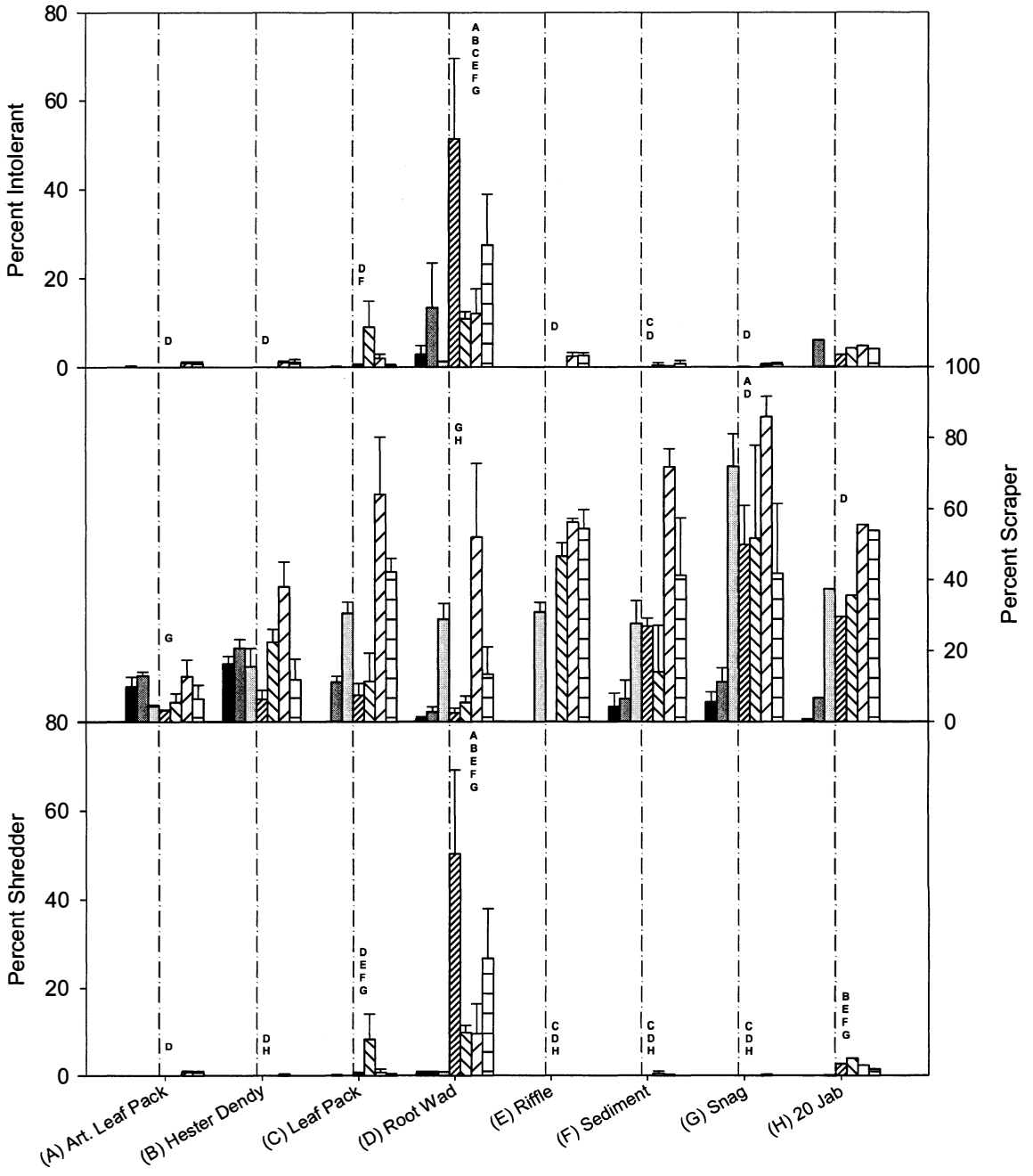
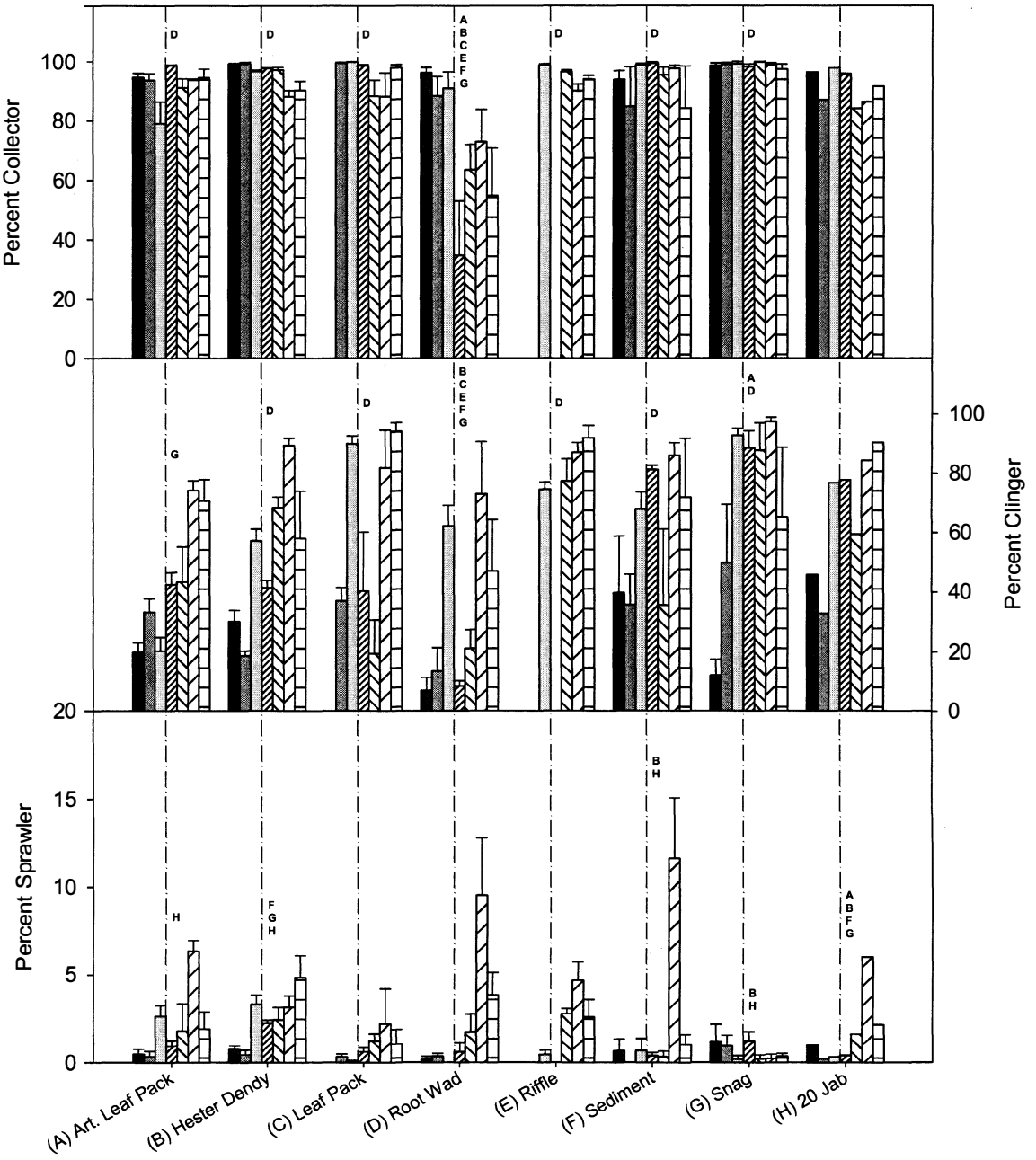


Figure 2. NMDS plot of macroinvertebrate assemblages from all sites separated by microhabitat type. Data was transformed to relative abundance. Plotted using Bray-Curtis dissimilarities with stress = 0.17734. PERMANOVA $p = 0.001$ with an R^2 value of 0.25. Microhabitat (represented by different symbols) positions are displayed using the average x and y axis values for each microhabitat types with x and y standard error bars. Each vector represents the most abundant taxa sampled. Numbers that are circled represent the distinct taxa driving the assemblage composition of a few microhabitat types (thin black lined circle for riffles, thick black line for artificial leaf packs and Hester Dendy samplers, and grey lined circle for root wads). Numbers representing certain taxa for each vector are in Appendix 1 with their taxonomic identification.


MBI
Percent EPT
Percent non-Hyd./Poly. EPT
Site 3
Site 5
Site 7
Site 9
Site 12
Site 14
Site 15
(A) Art. Leaf Pack
(B) Hester Dendy
(C) Leaf Pack
(D) Root Wad
(E) Riffle
(F) Sediment
(G) Snag
(H) 20 Jab


Richness
Simpson's Diversity
Percent Mayfly
0
10
20
30
40
0.0
0.2
0.4
0.6
0.8
1.0
1.2
F
D H
B F G
F G
A D E H
D E H
B F G
G
G
G
A B E
A B F G
E
E
E
(A) Art. Leaf Pack
(B) Hester Dendy
(C) Leaf Pack
(D) Root Wad
(E) Riffle
(F) Sediment
(G) Snag
(H) 20 Jab


Percent Intolerant
Percent Scraper
Percent Shredder
(A) Art. Leaf Pack
(B) Hester Dendy
(C) Leaf Pack
(D) Root Wad
(E) Riffle
(F) Sediment
(G) Snag
(H) 20 Jab


Percent Collector
Percent Clinger
Percent Sprawler
(A) Art. Leaf Pack
(B) Hester Dendy
(C) Leaf Pack
(D) Root Wad
(E) Riffle
(F) Sediment
(G) Snag
(H) 20 Jab

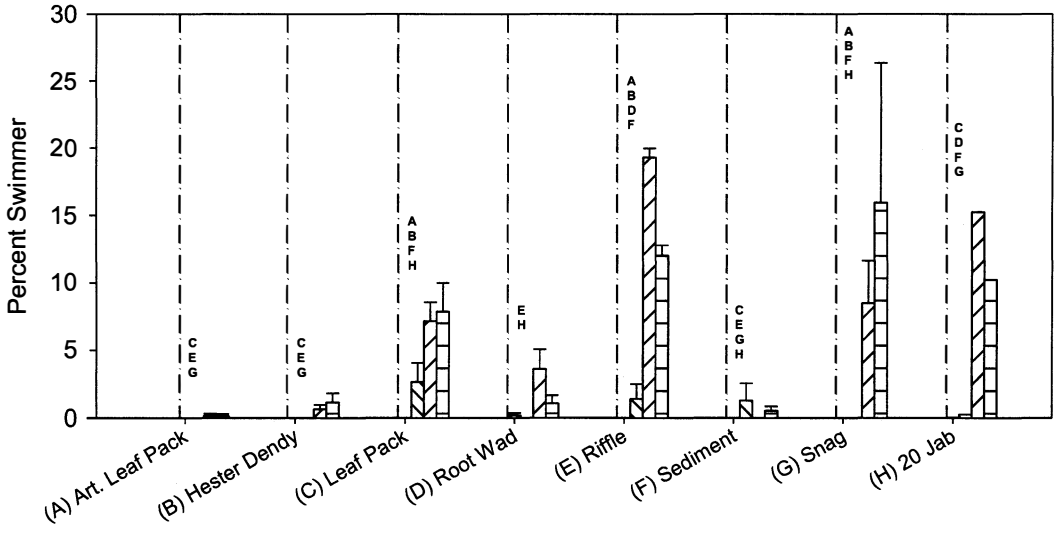


Figure 3. Bar plots displaying thirteen population indices (MBI, percent EPT, percent non-Hydropsychidae and Polycentropodidae EPT, richness, Shannon's diversity, scrappers, shredders, collectors, clingers, sprawlers, swimmers, mayflies, intolerant). Each bar grouping is separated by microhabitat sampled (artificial leaf pack, Hester Dendy sampler, leaf pack, root wad, riffle, sediment, snag, and 20 jab) with each individual bar representing a site sampled. Dotted lines represent the point in which the municipal effluent inters the stream downstream of site seven. Letters above each of the microhabitat types represent which microhabitats (or sample in the case of 20 jab) are significantly different from them (ex. Hester Dendy samplers (B) are significantly lower in percent swimmers than leaf packs (C), riffles (E), and snags (G)). P-values are summarized in Appendix 3 and Table 3.

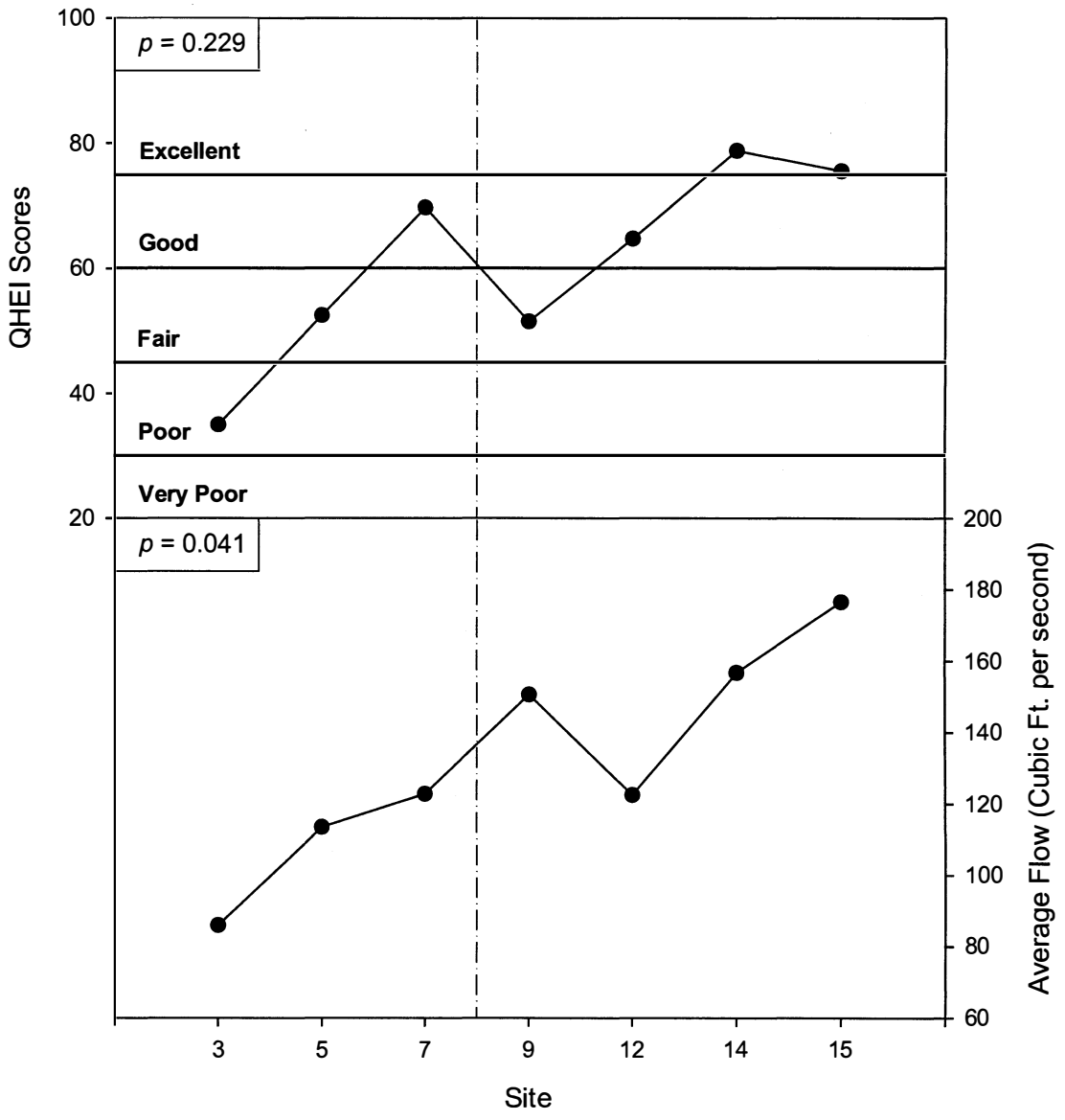


Figure 4. QHEI scores (top) and average flow (bottom) for each site. Dotted line represents the point the effluent inters the stream. Average flow was significantly (p = 0.041) higher downstream.

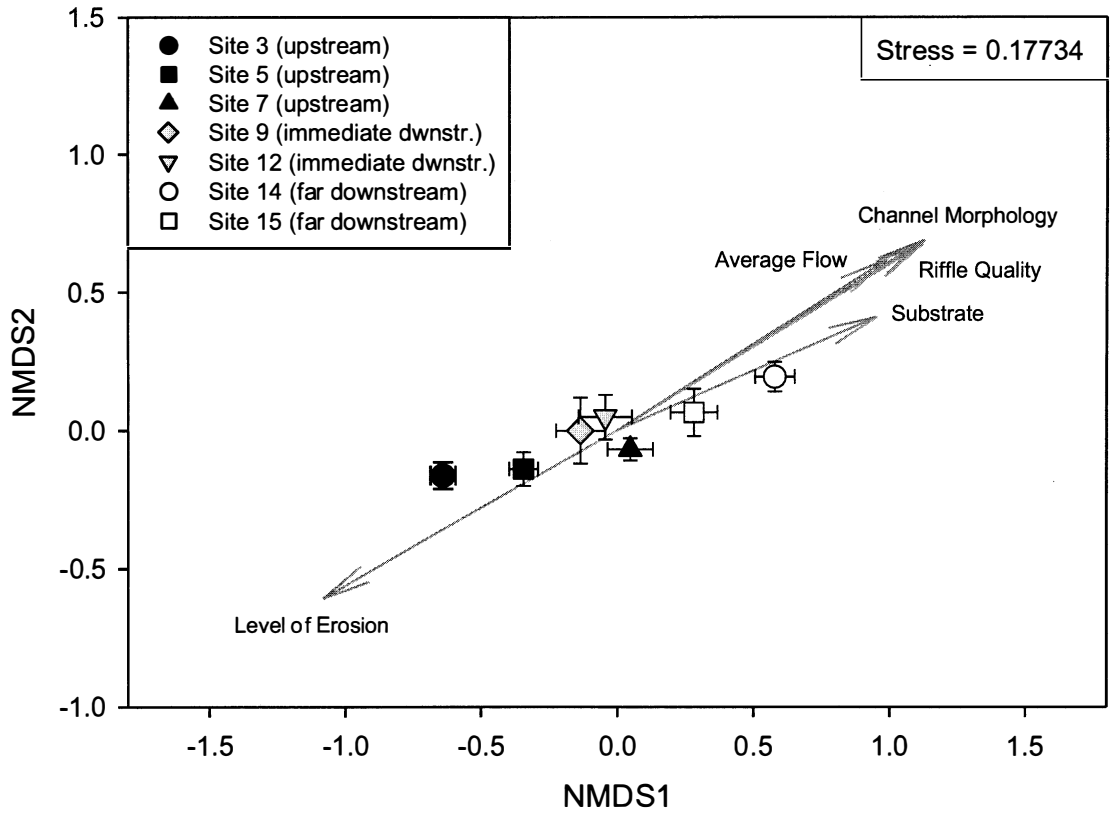


Figure 5. NMDS plot of macroinvertebrate assemblages sorted by the seven sampling sites. Data was transformed to relative abundance. Plotted using Bray-Curtis dissimilarities with stress = 0.17734. PERMANOVA results indicate that differences between site area assemblages (upstream, immediately downstream, and far downstream) that $p = 0.001$ with an R^2 value of 0.18. The plot shows seven different symbols each representing the site sampled. Their positions are displayed using the average x and y axis values for each site with x and y standard error bars. Physical variables (average flow, riffle quality, channel morphology, substrate, and level of erosion) are overlaid in vector form to show the relationship between physical features and site location.

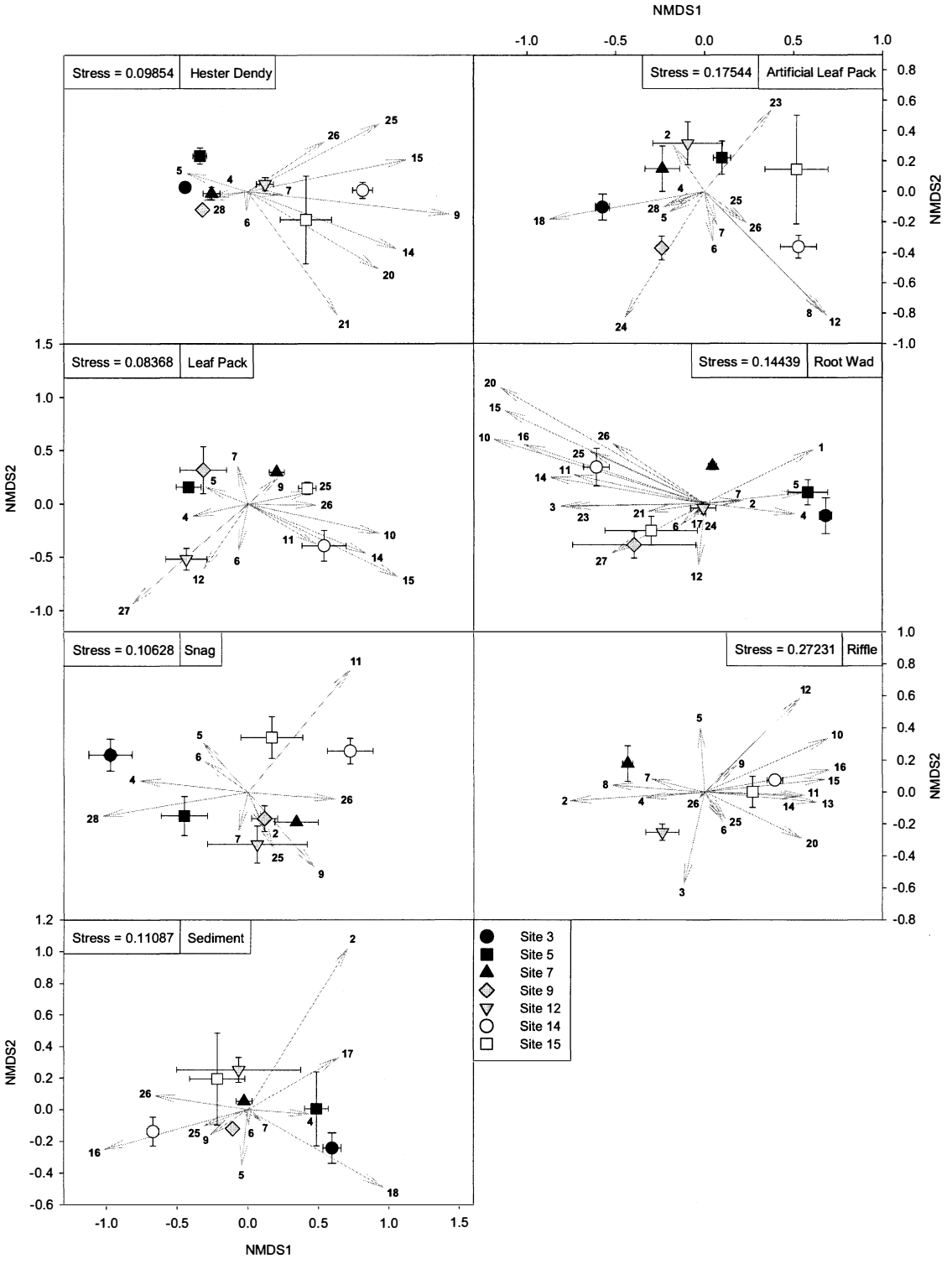

NMDS1
NMDS2
Stress = 0.09854
Hester Dendy
Stress = 0.17544
Artificial Leaf Pack
Stress = 0.08368
Leaf Pack
Stress = 0.14439
Root Wad
Stress = 0.10628
Snag
Stress = 0.27231
Riffle
Stress = 0.11087
Sediment
Site 3
Site 5
Site 7
Site 9
Site 12
Site 14
Site 15

Figure 6. NMDS plots displaying square-root transformed assemblage data by habitat type (artificial leaf packs, Hester Dendy samplers, leaf packs, root wads, riffles, snags, sediments) plotted using Bray-Curtis dissimilarities. There was a significant difference between sites within all microhabitats (PERMANOVA $p = 0.001$). In these plots important taxa are represented by vectors to show their prominence at certain sites. Taxa are labeled by a specific number that corresponds to their taxonomic identification Appendix 1.

APPENDIX

Appendix 1. List of (mostly) genus level taxa found during the time of sampling grouped by their higher taxonomic levels. Tolerance levels (Tv) for each group are displayed. Subfamily (*) and tribe (**) are shown in place of genus for the members of Chironomidae. Graph numbers for certain taxa are also listed.

Phylum	Class	Order	Family	Genus	ID code	Tv	Graph Number
Platyhelmnithes	Turbellaria				pla.tur	6	
Annelida	Oligochaeta				ann.oli	10	2
	Hirudinea				ann.hir	8	
Mollusca	Gastropoda		Lymnaeidae	Fossaria	gas.lym.fos	7	
			Planorbidae	Menetus	gas.pla.men	6.5	
			Physidae	Unknown	gas.phy.unk	8	17
			Pleuroceridae	Pleurocera	gas.ple.ple	7	18
			Ancylidae	Ferrissia	gas.anc.fer	7	
Arthropoda	Amphipoda		Gammaridae	Gammarus	amp.gam.gam	3	1
			Hyalellidae	Hyalella	amp.hya.hya	5	
	Isopoda		Asellidae	Caecidotea	iso.ase.cae	6	19
	Insecta	Coleoptera	Elmidae	Stenelmis	col.elm.ste	7	3
				Ancyronyx	col.elm.anc	2	
				Dubiraphia	col.elm.dub	5	
				Macronychus	col.elm.mac	2	
		Odonata	Aeshnidae	Boyeria	odo.aes.boy	3	
			Aeshnidae	Nasiaeschna	odo.aes.nas	2	
			Calopterygidae	Hetaerina	odo.cal.het	3	
			Coenagrionidae	Argia	odo.coe.arg	5	21
				Enallagma	odo.coe.ena	6	
			Corduliidae	Neurocordulia	odo.cor.neur	3	22
			Gomphidae	Dromogomphus	odo.gom.dro	4	23
			Macromiidae	Macromia	odo.mac.mac	3	24
		Diptera	Chaboridae	Chaoborus	dip.cha.cha	8	
			Ceratopogonidae	Atrichopogon	dip.cer.achp	2	
				Dasyhelea	dip.cer.das	5	
			Chironomidae	Tanypodinae*	dip.chi.tanyp	6	6
				Tanytarsini	dip.chi.tanyt	6	7
				Chironomini*	dip.chi.chi	8	4

		Orthocladiinae	dip.chi.oli	6	5
	Culicidae	Anopheles	dip.cul.ano	6	
	Empididae	Hemerodromia	dip.eph.hem	6	8
	Muscidae	Unknown	dip.musc.unk	8	
	Psychodidae	Unknown	dip.psy.unk	11	
	Simuliidae	Simulium	dip.sim.sim	6	9
	Tipulidae	Ormosia	dip.tip.orm	6.5	
Ephemeroptera	Baetidae	Acerpenna	eph.bae.acer	4	10
		Acentrella	eph.bae.acen	4	
		Baetis	eph.bae.bae	4	11
		Procloeon	eph.bae.pro	4	
	Heptageniidae	Heptagenia	eph.hep.hep	3	13
		Maccaffertium	eph.hep.mae	4	14
		Stenacron	eph.hep.stena	4	
		Unknown	eph.hep.unk	3	
	Caenidae	Caenis	eph.cae.cae	6	12
	Ephemeridae	Hexagenia	eph.eph.hex	6	
	Isonychiidae	Isonychia	eph.iso.iso	3	15
	Leptohyphidae	Tricorythodes	eph.lep.tri	5	16
	Unknown	Unknown	eph.unk	3	
Plecoptera	Unknown	Unknown	ple.unk	1.5	
Megaloptera	Corydalidae	Corydalus	mega.cory.cory	3	20
Trichoptera	Hydropsychidae	Hydropsyche	tri.hyd.che	6	25
		Cheumatopsyche	tri.hyd.hyd	5	26
	Hydroptilidae	Hydroptila	tri.hydropt.hyd	2	
		Mayatrichia	tri.hydropt.maya	1	
	Philopotamidae	Chimarra	tri.phi.chi	3	
	Polycentropodidae	Cyrnellus	tri.poly.cyn	5	28
		Polycentropus	tri.poly.poly	3	
	Leptoceridae	Nectopsyche	tri.lep.nec	3	27

Appendix 2. List of macroinvertebrates collected (transformed into estimated abundance) in fall of 2016 separated by microhabitat type. Taxa codes are in Appendix 1.

Art. Leaf Pack

	Sites with Replicates																				
	3			**5**			**7**			**9**			**12**			**14**			**15**		
Taxa Code	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
ann.hir																6					
ann.oli		12	8	90	40	13	130		117			15	9	30	8					20	
col.elm.dub																				4	
col.elm.ste				5		13							3			12				7	
dip.chi.chi	1290	762	923	505	570	489	244	394	270	990	1230	908	183	205	428	204	308	300	486	127	300
dip.chi.oli	330	72	38	85	60	35	24	27	9	60	80	45	36		45	24	98	10	18		30
dip.chi.tanyp	20	6				9	7	23	15	30	20	8	3		60	54	83	100		17	70
dip.chi.tanyt	420	72	83	160	230	245	27	83	81	590	1090	323	246		503	762	765	1400	744	397	1730
dip.eph.hem																	8	10			
dip.sim.sim													6		8						40
eph.cae.cae																6					10
eph.eph.hex																				4	
eph.hep.mae																6				7	
eph.hep.stena	10	6	8										3	3							
eph.hep.unk			8																		
eph.iso.iso																	8		6		
eph.lep.tri																6	8	50			
gas.lym.fos					5																
gas.phy.unk					5			8													
gas.ple.ple	30	54	53																		
odo.cce.arg							10	4					9	13	8				12	4	
odo.gom.dro				5	10	5							3		8				6	17	
odo.mac.mac											1										
pla.tur								8													
tri.hyd.che			8		10	5		4	3				3	5		36	23	60			30
tri.hyd.hyd			45	35	55	86			12			8	15	5		48	255	170		7	320
tri.lep.nec																18	8	10	12	7	
tri.poly.cyn	110	66	143	30	70	26	27	79	30	280	160	188	30	48	165	48	30	30	18	24	20

Hester Dendy

Sites with Replicates

	3			5			7			9			12			14			15		
Taxa Code	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
ann.oli																			4		
col.elm.ste															6						10
dip.chi.chi	308	550	262	1043	670	966	233	198	290	654	660	672	450	293	288	72	42	105	330	416	200
dip.chi.oli	124	105	96	233	165	414	102	216	50	54	173	42	83	68	48	4			15	5	10
dip.chi.tanyp	4	5	6	15		6	23	48	25	30	30	30	30	23	48	12	6	20	49	22	80
dip.chi.tanyt	23	30	17	83	35	114	23	126	75	108	150	84	353	495	324	390	318	375	225	249	1810
dip.eph.hem																4					
dip.sim.sim																4	6				
eph.bae.bae																	6				
eph.cae.cae																				5	10
eph.hep.mae																68	60	125	19	48	30
eph.hep.stena																	12	5	4		
eph.iso.iso																	6	10	8		70
eph.lep.tri																15	36		4	5	10
gas.phy.unk				8																	
mega.cory.cory																1				1	1
odo.coe.arg													8		6	4	6	5	8	43	
odo.gom.dro				8	1																
pla.tur																		5			
tri.hyd.che				23		18									6	27	120	65			110
tri.hyd.hyd	4			68	20	30	4	66	20				150	345	258	117	504	305			640
tri.hydropt.maya																	12				
tri.lep.nec																4		5			
tri.poly.cyn	233	185	131	68	170	174	330	474	530	318	518	510	435	285	270	42	54	35	57	35	

Leaf Pack

Sites with Replicates

	5			7			9			12			14			15		
Taxa Code	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
col.elm.mac												1						
col.elm.ste													9					
dip.cer.achp										2								
dip.cha.cha		5																
dip.chi.chi	698	790	668	360	240	225	1	200	450	184	66	86	326	72	60	8	14	30
dip.chi.oli	113	115	188	40	10				8			1	5		6		14	23
dip.chi.tanyp			8					3	15	3		1	48		6	3		13
dip.chi.tanyt	593	385	413	2260	1830	788	5	178	698	3	15	1	69	60	42	195	250	280
dip.eph.hem				10														3
dip.psy.unk		20						3										
dip.sim.sim	38	5	23	180	280	60		5	60	4			9		6	53	44	18
eph.bae.acer															12			3
eph.bae.bae										13	2	1	73	48	78	28	90	35
eph.cae.cae											1							
eph.hep.mae				10									159	30	66	3	7	8
eph.hep.stena										3			22					
eph.iso.iso													9	12	18			
eph.lep.tri													9					
gas.phy.unk							1			3								
mega.cory.cory													5					
odo.coe.arg										8	1	1	5					
odo.mac.mac													1					
tri.hyd.che	15		15	350	320	30			98				56	48	66	10		
tri.hyd.hyd	30	10	8	1700	1220	368		26	203	7	32	2	108	1110	930	250	324	175
tri.lep.nec		5						5		10	2	23	22					
tri.phi.chi																	4	
tri.poly.cyn		5	23			8					1		5					
tri.poly.poly																		5

Riffle

Sites with Replicates

	7			12			14			15		
Taxa Code	1	2	3	1	2	3	1	2	3	1	2	3
ann.oli	10											
col.elm.ste	10			20	52	40	10			120		60
dip.chi.chi	320	143	370	280	161	105	140	70	60	130	80	100
dip.chi.oli	60	120	70			10	140	250	120	40	270	10
dip.chi.tanyp			10	30	14	20	30	70	40	10	20	50
dip.chi.tanyt	600	420	1000	700	77	195	160	120	90	1030	680	700
dip.eph.hem		8		30		5	10					
dip.sim.sim	20	8	10		3		60	30	50	120	80	40
eph.bae.acen											10	
eph.bae.acer							80	70	10		30	
eph.bae.bae					17	5	240	360	580	460	220	440
eph.cae.cae											10	
eph.hep.hep									20	70	10	40
eph.hep.mae					3	10	270	100	180	320	50	170
eph.hep.stena	1									10		10
eph.iso.iso							60	20	90	150	30	50
eph.lep.tri							30	20	190	40	70	50
gas.phy.unk	10											10
iso.ase.cae		8										
mega.cory.cory										3		2
odo.coe.arg	1											
odo.gom.dro	1				1							
tri.hyd.che	210			200	14	35	110	190	440	170	60	250
tri.hyd.hyd	460	210	460	1350	131	330	780	960	1510	3120	710	1760
tri.hydropt.hyd									20			
tri.poly.cyn	50		60	100	3	20						

Root Wad

Sites with Replicates

	3			5			7			9			12			14			15		
Taxa Code	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
amp.gam.gam	7				555	90	2	2	10		19						20	10			
ann.oli	2				23	75				3											5
col.elm.anc													8								
col.elm.dub														30		40				7	5
col.elm.mac														10							
col.elm.ste					8					3		2				110	80	20		3	5
dip.cer.achp													16								
dip.chi.chi	69	372	145	58	743	983	128	92	770		420	55	938	1060	169	600	170	70	330	114	30
dip.chi.oli	2	2			60	68			30								10		30	3	
dip.chi.tanyp		2			8	8						4	15	30	4	130	10	20	50	5	20
dip.chi.tanyt	15	8	4	17	90	53	76	66	1300		34	7	38	610	102	460	90	130	1170	22	245
dip.cul.ano											4										
dip.eph.hem																20			10	3	
dip.sim.sim								2	20												
eph.bae.acer																		20			5
eph.bae.bae																30	20	100	20		5
eph.cae.cae												2	15								
eph.hep.mae																100	360	340	30	20	190
eph.hep.stena							2					2				10	20				25
eph.iso.iso																	110	70			10
eph.lep.tri																400	300	100			40
gas.anc.fer										3											
gas.phy.unk						218	74	3	160	3	42	104	158	460	192					5	
gas.pla.men			1																		
gas.ple.ple	1		2																		
iso.ase.cae	4				165	15															
mega.cory.cory																		1			
odo.aes.boy										1											2
odo.aes.nas																		1			
odo.cal.het																				3	3
odo.coe.arg					8	15			10		19	4	23	310	72	700	10	50	10	28	75

odo.coe.ena											4										3	
odo.cor.neur									1						4		1					
odo.gom.dro																1						
odo.mac.mac						1					1					5						
pla.tur																	20					
tri.hyd.che								3	170	3				30		10	60	100				
tri.hyd.hyd							82	105	1070	13	4		8	220	42	140	1660	2160				50
tri.lep.nec		2	2			23	4	2	30	265	139	162	135	240	90	860	90	110	140	189		245
tri.poly.cyn	1	2		7	23	15	38	18	40	18	19	9	83	50	15	120	40	10				

Snag

Sites with Replicates

	3			5			7			9			12			14			15		
Taxa Code	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
ann.oli						3		30		1											
col.elm.ste																	2				
dip.cer.achp																					1
dip.cer.das	1				9																
dip.chi.chi	156	26	54	45	240	185	36		110	16	140	30	35	22	36		3	4	4	1	54
dip.chi.oli	9	3		15	75	5		10	5	2	40	30					3	4	12	2	6
dip.chi.tanyp		1			3	2				2	10	20		5			1				4
dip.chi.tanyt	6	2	1	353	225	43	84	180	295	36	1460	370	68	185	192	2	10	12	49	4	27
dip.eph.hem								10											4		
dip.sim.sim				3				10			110	10		5	6				4		
dip.tip.crm					6																
eph.bae.acer																				1	
eph.bae.bae																18	20	22	98	4	7
eph.bae.pro																	1				
eph.hep.hep																	1	2			1
eph.hep.mae																		4	4		2
gas.phy.unk					3																
gas.ple.ple	2																				
pla.tur																		2			
ple.unk											10										
tri.hyd.che				5		2	3	10	15	1	170	10	3	5		2	1		4		2
tri.hyd.hyd	4			63	6	3	375	1440	545	33	1190	1130		549	1230	296	100	388	657	2	59
tri.lep.nec																		2			
tri.poly.cyn	3		15		3	4			35		10	10	8					2			2

Sediments

Sites with Replicates

	3			5			7			9			12			14			15		
Taxa Code	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
ann.hir									1	8						3				1	
ann.oli				9										1						17	
col.elm.dub																				1	
col.elm.ste							1	2					1							1	
dip.chi.chi	42	10	210	122	256	94	30	45	72	150	290	143	7	11		53	45	10	14	9	10
dip.chi.oli	2	2			10	64	6	1	3	120	100	75				3	55	62	6		35
dip.chi.tanyp			2				2			8		8				3					
dip.chi.tanyt	169	3	68	82	104	214	42	47	142	848	1110	788	22	2	1	33	125	52	60	10	330
dip.eph.hem																	10	5	1		5
dip.musc.unk			4																		
dip.sim.sim									7	8	20	38									5
eph.bae.acer													2						1		
eph.bae.bae																			1		5
eph.eph.hex																				1	
eph.hep.mae													1					10	2		10
eph.lep.tri																80	55	50	1		15
eph.unk																				1	
gas.anc.fer									1												
gas.lym.fos		1		18																	
gas.phy.unk				119	4	10		1	1								5				
gas.ple.ple	4	1	8																		
iso.ase.cae				2																	
tri.hyd.che				2			3	3	2	30		45					15	7	8		10
tri.hyd.hyd							10	31	147	195	530	308	19		7	408	1050	150	88	2	570
tri.lep.nec																3					
tri.poly.cyn			2				2	2	1												

Appendix 3. MANOVA univariate *p*-values. Combined all sites for each microhabitat type and then were compared to each other for each of the indices analyzed.

Compared Habitat types	MBI	Pct. EPT	Pct. Non-hyd./poly. EPT	Sim. Diversity	Richness	Pct. Scraper	Pct. Shredder	Pct. Collector	Pct. Clinger	Pct. Sprawler	Pct. Swimmer	Pct. Mayfly	Pct. Intolerant
HD-a_leaf	0.216	0.022	1	1	1	1	1	1	1	1	1	1	1
leaf-a_leaf	0.305	1	0.023	1	1	1	1	1	1	1	<0.001	0.803	1
riff-a_leaf	0.001	0.233	0.042	1	1	0.146	1	1	0.569	1	<0.001	0.012	1
root-a_leaf	0.001	1	<0.001	1	1	1	<0.001	<0.001	0.052	1	1	1	<0.001
sed-a_leaf	0.097	1	1	1	0.042	1	1	1	0.392	1	1	1	1
snag-a_leaf	<0.001	0.009	1	0.016	0.065	0.024	1	1	0.044	1	0.002	1	1
leaf-HD	1	1	0.002	1	1	1	0.064	1	1	0.094	0.001	0.324	0.881
riff-HD	1	1	0.006	1	0.298	1	1	1	1	1	<0.001	0.004	1
root- HD	1	1	<0.001	1	0.040	1	<0.001	<0.001	<0.001	1	1	1	<0.001
sed-HD	1	0.008	1	1	1	1	1	1	1	0.011	1	1	1
snag-HD	1	1	1	<0.001	1	1	1	1	1	0.019	0.227	1	1
riff-leaf	1	1	1	1	0.549	1	0.003	1	1	1	1	1	1
root-leaf	1	1	<0.001	1	0.107	1	<0.001	<0.001	<0.001	1	0.086	1	<0.001
sed-leaf	1	1	0.011	1	1	1	0.016	1	1	1	<0.001	1	0.005
snag-leaf	1	0.918	0.038	0.487	1	1	0.015	1	1	1	1	1	0.056
root-riff	1	1	0.001	1	1	0.072	<0.001	<0.001	<0.001	1	<0.001	1	<0.001
sed-riff	1	0.108	0.022	1	0.001	1	1	1	1	1	<0.001	0.020	0.499
snag-riff	1	1	0.063	<0.001	0.001	1	1	1	1	1	0.072	0.020	1
sed-root	1	1	<0.001	1	<0.001	1	<0.001	<0.001	<0.001	1	1	1	<0.001
snag-root	1	1	<0.001	0.440	<0.001	0.009	<0.001	<0.001	<0.001	1	1	1	<0.001
snag-sed	1	0.003	1	0.189	1	1	1	1	1	1	0.044	1	1

Appendix 4. MANOVA univariate *p*-values when comparing every site to each other for each of the indices analyzed.

Indices	Compared Sites	Art. Leaf	Hester Dendy	Leaf	Riffle	Root Wad	Sediment	Snag
MBI	5-3	0.999	0.717	N/A	N/A	0.891	1	0.509
	7-3	0.739	0.039	N/A	N/A	0.403	0.873	0.001
	9-3	0.842	0.997	N/A	N/A	0.019	0.731	0.005
	12-3	0.693	0.056	N/A	N/A	0.727	0.892	0.005
	14-3	0.009	0.001	N/A	N/A	0.013	0.217	<0.001
	15-3	0.036	0.703	N/A	N/A	0.023	0.919	0.002
	7-5	0.477	0.002	0.001	N/A	0.963	0.814	0.043
	9-5	0.976	0.400	0.432	N/A	0.149	0.654	0.139
	12-5	0.909	0.003	0.988	N/A	1	0.836	0.148
	14-5	0.020	<0.001	<0.001	N/A	0.108	0.176	0.006
	15-5	0.081	0.085	<0.001	N/A	0.179	0.871	0.054
	9-7	0.144	0.105	0.033	N/A	0.535	1	0.992
	12-7	0.088	1	0.004	0.886	0.997	1	0.989
	14-7	0.001	0.377	0.235	0.002	0.429	0.834	0.927
	15-7	0.002	0.465	0.407	0.005	0.602	1	1
	12-9	1	0.146	0.773	N/A	0.255	1	1
	14-9	0.088	0.002	0.001	N/A	1	0.940	0.599
	15-9	0.308	0.944	0.001	N/A	1	0.999	0.997
	14-12	0.143	0.288	<0.001	0.004	0.191	0.812	0.578
	15-12	0.449	0.576	<0.001	0.013	0.301	1	0.996
	15-14	0.982	0.014	0.998	0.751	1	0.771	0.885
Pct. EPT	5-3	0.998	0.146	N/A	N/A	0.867	1	0.992
	7-3	1	0.095	N/A	N/A	0.001	0.050	0.018
	9-3	0.998	0.951	N/A	N/A	<0.001	0.082	0.056
	12-3	0.889	0.431	N/A	N/A	0.016	0.190	0.078
	14-3	0.638	0.084	N/A	N/A	<0.001	0.008	0.008
	15-3	0.999	0.454	N/A	N/A	0.001	0.039	0.033
	7-5	0.999	0.001	0.089	N/A	0.010	0.048	0.061
	9-5	1	0.027	1	N/A	0.003	0.079	0.174
	12-5	0.992	0.004	0.276	N/A	0.143	0.183	0.234
	14-5	0.891	0.001	0.011	N/A	<0.001	0.007	0.028
	15-5	0.958	0.983	0.039	N/A	0.014	0.037	0.109
	9-7	0.998	0.418	0.085	N/A	0.986	1	0.995
	12-7	0.894	0.945	0.973	0.140	0.745	0.983	0.979
	14-7	0.647	1	0.805	0.003	0.616	0.939	0.999
	15-7	0.999	0.003	0.995	0.013	1	1	1

	12-9	0.994	0.927	0.266	N/A	0.331	0.998	1
	14-9	0.903	0.382	0.010	N/A	0.955	0.837	0.933
	15-9	0.951	0.107	0.037	N/A	0.963	0.999	1
	14-12	0.999	0.926	0.401	0.089	0.072	0.561	0.866
	15-12	0.669	0.016	0.813	0.378	0.826	0.961	0.999
	15-14	0.391	0.002	0.970	0.707	0.523	0.970	0.985
Pct. Non-Hyd./Poly. EPT	5-3	0.004	1	N/A	N/A	0.983	1	1
	7-3	0.004	1	N/A	N/A	0.942	1	1
	9-3	0.004	1	N/A	N/A	0.001	1	0.875
	12-3	0.712	1	N/A	N/A	0.057	0.601	1
	14-3	0.628	<0.001	N/A	N/A	0.003	<0.001	<0.001
	15-3	0.986	<0.001	N/A	N/A	0.002	0.006	<0.001
	7-5	1	1	1	N/A	0.567	1	1
	9-5	1	1	1	N/A	<0.001	1	0.875
	12-5	0.068	1	0.007	N/A	0.014	0.601	1
	14-5	<0.001	<0.001	0.003	N/A	0.001	<0.001	<0.001
	15-5	0.001	<0.001	0.010	N/A	0.001	0.006	<0.001
	9-7	1	1	0.999	N/A	0.005	1	0.875
	12-7	0.068	1	0.006	0.227	0.297	0.601	1
	14-7	<0.001	<0.001	0.003	0.001	0.022	<0.001	<0.001
	15-7	0.001	<0.001	0.008	0.002	0.015	0.006	<0.001
	12-9	0.068	1	0.011	N/A	0.303	0.601	0.875
	14-9	<0.001	<0.001	0.005	N/A	1	<0.001	<0.001
	15-9	0.001	<0.001	0.015	N/A	0.997	0.006	<0.001
	14-12	0.067	<0.001	0.995	0.010	0.714	0.010	<0.001
	15-12	0.306	<0.001	1	0.027	0.589	0.135	<0.001
	15-14	0.958	<0.001	0.978	0.879	1	0.746	0.881
Sim. Diversity	5-3	0.163	0.772	N/A	N/A	0.073	0.946	0.773
	7-3	0.259	0.761	N/A	N/A	0.014	0.439	0.981
	9-3	0.594	1	N/A	N/A	0.158	0.887	0.441
	12-3	0.259	0.026	N/A	N/A	0.019	0.997	0.978
	14-3	0.475	0.134	N/A	N/A	0.023	0.998	0.994
	15-3	0.997	0.996	N/A	N/A	0.026	0.709	0.336
	7-5	1	0.121	0.997	N/A	0.962	0.938	0.994
	9-5	0.954	0.612	1	N/A	0.999	1	0.996
	12-5	1	0.002	0.730	N/A	0.986	0.708	0.995
	14-5	0.986	0.010	0.938	N/A	0.993	0.999	0.418
	15-5	0.381	0.434	0.985	N/A	0.997	0.997	0.981
	9-7	0.993	0.891	0.994	N/A	0.803	0.976	0.874
	12-7	1	0.302	0.482	0.973	1	0.201	1
	14-7	0.999	0.803	0.758	0.405	1	0.732	0.776

	15-7	0.542	0.973	1	0.952	1	0.999	0.775
	12-9	0.993	0.043	0.773	N/A	0.879	0.600	0.883
	14-9	1	0.210	0.958	N/A	0.915	0.992	0.181
	15-9	0.891	1	0.974	N/A	0.937	1	1
	14-12	0.999	0.960	0.996	0.628	1	0.926	0.764
	15-12	0.543	0.076	0.375	1	1	0.395	0.788
	15-14	0.800	0.333	0.642	0.681	1	0.935	0.128
Richness	5-3	0.989	0.131	N/A	N/A	0.994	1	0.470
	7-3	1	0.923	N/A	N/A	0.696	0.093	0.982
	9-3	0.510	0.998	N/A	N/A	0.599	0.831	0.369
	12-3	0.980	0.131	N/A	N/A	0.459	0.670	1
	14-3	0.478	<0.001	N/A	N/A	0.013	0.311	0.484
	15-3	0.926	<0.001	N/A	N/A	0.162	0.064	0.261
	7-5	0.959	0.584	0.994	N/A	0.958	0.082	0.889
	9-5	0.191	0.052	0.790	N/A	0.913	0.792	1
	12-5	1	1	0.962	N/A	0.813	0.715	0.535
	14-5	0.869	<0.001	0.584	N/A	0.040	0.278	1
	15-5	1	0.013	0.985	N/A	0.406	0.056	0.999
	9-7	0.638	0.671	0.970	N/A	1	0.607	0.804
	12-7	0.938	0.584	0.769	0.866	0.999	0.005	0.992
	14-7	0.364	<0.001	0.317	0.119	0.200	0.985	0.899
	15-7	0.844	0.001	0.845	0.033	0.903	1	0.670
	12-9	0.165	0.052	0.358	N/A	1	0.112	0.427
	14-9	0.022	<0.001	0.101	N/A	0.257	0.952	1
	15-9	0.107	<0.001	0.435	N/A	0.951	0.483	1
	14-12	0.903	<0.001	0.951	0.333	0.363	0.020	0.550
	15-12	1	0.013	1	0.096	0.988	0.003	0.309
	15-14	0.970	0.408	0.907	0.794	0.768	0.951	0.999
Pct. Scaper	5-3	0.986	0.993	N/A	N/A	0.994	1	0.947
	7-3	0.704	1	N/A	N/A	0.023	0.495	0.146
	9-3	0.387	0.277	N/A	N/A	0.897	0.491	0.280
	12-3	0.794	0.976	N/A	N/A	0.502	0.827	0.825
	14-3	0.998	0.272	N/A	N/A	0.008	0.132	0.101
	15-3	0.778	0.900	N/A	N/A	0.196	0.384	0.293
	7-5	0.298	0.951	0.787	N/A	0.074	0.626	0.572
	9-5	0.124	0.097	0.619	N/A	0.998	0.622	0.807
	12-5	0.375	1	0.999	N/A	0.856	0.917	1
	14-5	1	0.611	0.244	N/A	0.027	0.190	0.448
	15-5	0.359	0.560	0.518	N/A	0.479	0.506	0.823
	9-7	0.997	0.423	0.110	N/A	0.175	1	0.999
	12-7	1	0.898	0.570	0.031	0.497	0.996	0.765
	14-7	0.418	0.168	0.883	0.003	0.997	0.962	1
	15-7	1	0.976	0.996	0.005	0.870	1	0.999

	12-9	0.988	0.070	0.828	N/A	0.986	0.996	0.937
	14-9	0.187	0.004	0.018	N/A	0.068	0.963	0.994
	15-9	0.990	0.875	0.050	N/A	0.771	1	1
	14-12	0.509	0.716	0.136	0.337	0.234	0.728	0.642
	15-12	1	0.456	0.323	0.523	0.991	0.980	0.946
	15-14	0.491	0.040	0.990	0.979	0.570	0.989	0.992
Pct. Shredder	5-3	1	1	N/A	N/A	0.988	1	1
	7-3	1	1	N/A	N/A	0.974	1	1
	9-3	1	1	N/A	N/A	0.002	1	1
	12-3	1	1	N/A	N/A	0.089	1	1
	14-3	0.003	0.028	N/A	N/A	0.182	0.527	0.527
	15-3	0.035	1	N/A	N/A	0.013	1	1
	7-5	1	1	0.988	N/A	0.690	1	1
	9-5	1	1	1	N/A	<0.001	1	1
	12-5	1	1	0.084	N/A	0.024	1	1
	14-5	0.003	0.028	0.999	N/A	0.052	0.527	0.527
	15-5	0.035	1	1	N/A	0.003	1	1
	9-7	1	1	0.958	N/A	0.008	1	1
	12-7	1	1	0.030	NaN	0.339	1	1
	14-7	0.003	0.028	0.919	NaN	0.569	0.527	0.527
	15-7	0.035	1	0.971	NaN	0.059	1	1
	12-9	1	1	0.117	N/A	0.357	1	1
	14-9	0.003	0.028	1	N/A	0.191	0.527	0.527
	15-9	0.035	1	1	N/A	0.918	1	1
	14-12	0.003	0.028	0.149	NaN	0.999	0.527	0.527
	15-12	0.035	1	0.105	NaN	0.920	1	1
	15-14	0.801	0.028	1	NaN	0.728	0.527	0.527
Pct. Collector	5-3	1	1	N/A	N/A	1	0.965	1
	7-3	0.058	0.899	N/A	N/A	1	1	0.998
	9-3	0.984	0.992	N/A	N/A	0.022	0.999	1
	12-3	0.990	0.965	N/A	N/A	0.744	1	0.935
	14-3	1	0.002	N/A	N/A	0.934	1	1
	15-3	1	0.013	N/A	N/A	0.402	0.949	1
	7-5	0.082	0.906	1	N/A	1	0.840	1
	9-5	0.952	0.994	0.961	N/A	0.039	0.817	0.995
	12-5	0.998	0.969	0.527	N/A	0.894	0.898	0.990
	14-5	1	0.002	0.482	N/A	0.989	0.877	1
	15-5	1	0.013	1	N/A	0.581	1	0.995
	9-7	0.014	0.999	0.951	N/A	0.032	1	0.979
	12-7	0.190	1	0.502	0.694	0.850	1	0.998
	14-7	0.078	0.015	0.458	0.011	0.977	1	1
	15-7	0.061	0.100	1	0.166	0.517	0.802	0.977
	12-9	0.751	1	0.926	N/A	0.275	1	0.834

	14-9	0.959	0.006	0.900	N/A	0.137	1	0.999
	15-9	0.981	0.043	0.991	N/A	0.585	0.777	1
	14-12	0.998	0.009	1	0.050	0.999	1	0.971
	15-12	0.992	0.065	0.659	0.622	0.995	0.868	0.828
	15-14	1	0.927	0.613	0.275	0.931	0.844	0.999
Pct. Clinger	5-3	0.527	0.302	N/A	N/A	0.952	1	0.016
	7-3	1	0.033	N/A	N/A	0.010	0.731	<0.001
	9-3	0.131	0.593	N/A	N/A	0.995	0.475	<0.001
	12-3	0.177	0.004	N/A	N/A	0.470	0.940	<0.001
	14-3	0.002	<0.001	N/A	N/A	0.006	0.402	<0.001
	15-3	0.003	0.048	N/A	N/A	0.056	0.747	<0.001
	7-5	0.527	0.001	0.098	N/A	0.058	0.737	0.158
	9-5	0.949	0.015	0.563	N/A	1	0.481	0.217
	12-5	0.981	<0.001	0.218	N/A	0.945	0.943	0.236
	14-5	0.047	<0.001	0.187	N/A	0.036	0.408	0.114
	15-5	0.074	0.001	0.074	N/A	0.275	0.753	0.430
	9-7	0.131	0.517	0.799	N/A	0.031	0.999	1
	12-7	0.177	0.911	0.002	0.983	0.297	0.999	1
	14-7	0.002	0.125	0.998	0.336	1	0.996	1
	15-7	0.003	1	1	0.140	0.958	1	0.991
	12-9	1	0.101	0.014	N/A	0.815	0.959	1
	14-9	0.245	0.004	0.951	N/A	0.019	1	1
	15-9	0.351	0.636	0.709	N/A	0.159	0.999	0.999
	14-12	0.184	0.590	0.003	0.506	0.198	0.925	0.999
	15-12	0.270	0.829	0.001	0.228	0.804	0.999	0.999
	15-14	1	0.088	0.991	0.909	0.875	0.995	0.967
Pct. Sprawler	5-3	0.991	0.538	N/A	N/A	0.963	0.949	1
	7-3	0.703	0.317	N/A	N/A	0.965	1	0.954
	9-3	0.972	0.672	N/A	N/A	0.951	0.999	0.995
	12-3	1	0.647	N/A	N/A	0.200	0.949	0.957
	14-3	0.285	0.379	N/A	N/A	0.006	0.044	0.960
	15-3	0.995	0.114	N/A	N/A	0.042	0.976	1
	7-5	0.327	0.014	0.972	N/A	0.552	0.947	0.960
	9-5	0.708	0.045	1	N/A	1	0.772	0.994
	12-5	0.920	0.042	0.872	N/A	0.641	1	0.963
	14-5	0.095	0.017	0.992	N/A	0.031	0.007	0.965
	15-5	0.838	0.004	0.999	N/A	0.198	0.550	1
	9-7	0.991	0.993	0.929	N/A	0.518	0.999	0.698
	12-7	0.897	0.995	0.475	0.087	0.043	0.947	1
	14-7	0.982	1	0.777	0.033	0.001	0.044	1
	15-7	0.957	0.993	0.869	0.125	0.008	0.976	0.960
	12-9	0.999	1	0.937	N/A	0.676	0.772	0.706
	14-9	0.748	0.998	0.999	N/A	0.035	0.097	0.714

	15-9	1	0.832	1	N/A	0.218	1	0.994
	14-12	0.482	0.999	0.993	0.899	0.454	0.007	1
	15-12	1	0.851	0.973	0.994	0.962	0.550	0.963
	15-14	0.604	0.982	1	0.783	0.924	0.184	0.965
Pct. Swimmer	5-3	1	1	N/A	N/A	1	1	1
	7-3	1	1	N/A	N/A	1	1	1
	9-3	1	1	N/A	N/A	0.929	1	1
	12-3	1	1	N/A	N/A	1	0.713	1
	14-3	0.824	0.248	N/A	N/A	0.004	1	<0.001
	15-3	0.837	0.146	N/A	N/A	0.159	0.420	<0.001
	7-5	1	1	1	N/A	1	1	1
	9-5	1	1	1	N/A	0.929	1	1
	12-5	1	1	<0.001	N/A	1	0.713	1
	14-5	0.824	0.248	<0.001	N/A	0.004	1	<0.001
	15-5	0.837	0.146	<0.001	N/A	0.159	0.420	<0.001
	9-7	1	1	1	N/A	0.929	1	1
	12-7	1	1	<0.001	0.109	1	0.713	1
	14-7	0.824	0.248	<0.001	<0.001	0.004	1	<0.001
	15-7	0.837	0.146	<0.001	0.001	0.159	0.420	<0.001
	12-9	1	1	<0.001	N/A	0.929	0.713	1
	14-9	0.824	0.248	<0.001	N/A	0.027	1	<0.001
	15-9	0.837	0.146	<0.001	N/A	0.644	0.420	<0.001
	14-12	0.824	0.248	0.056	0.007	0.004	0.713	<0.001
	15-12	0.837	0.146	0.037	0.020	0.159	0.998	<0.001
	15-14	1	1	1	0.844	0.410	0.420	0.797
Pct. Mayfly	5-3	0.003	1	N/A	N/A	1	1	1
	7-3	0.003	1	N/A	N/A	0.980	1	1
	9-3	0.003	1	N/A	N/A	0.946	1	1
	12-3	0.683	1	N/A	N/A	0.951	0.600	1
	14-3	0.924	<0.001	N/A	N/A	<0.001	<0.001	<0.001
	15-3	1	<0.001	N/A	N/A	0.003	0.006	<0.001
	7-5	1	1	0.896	N/A	0.980	1	1
	9-5	1	1	1	N/A	0.946	1	1
	12-5	0.056	1	0.001	N/A	0.951	0.600	1
	14-5	<0.001	<0.001	<0.001	N/A	<0.001	<0.001	<0.001
	15-5	0.003	<0.001	<0.001	N/A	0.003	0.006	<0.001
	9-7	1	1	0.896	N/A	1	1	1
	12-7	0.056	1	0.004	0.227	1	0.600	1
	14-7	<0.001	<0.001	<0.001	0.001	0.002	<0.001	<0.001
	15-7	0.003	<0.001	<0.001	0.002	0.014	0.006	<0.001
	12-9	0.056	1	0.001	N/A	1	0.600	1
	14-9	<0.001	<0.001	<0.001	N/A	0.002	<0.001	<0.001
	15-9	0.003	<0.001	<0.001	N/A	0.020	0.006	<0.001

	14-12	0.173	<0.001	0.076	0.010	0.002	0.010	<0.001
	15-12	0.662	<0.001	0.404	0.027	0.019	0.134	<0.001
	15-14	0.934	<0.001	0.863	0.883	0.895	0.756	0.662
Pct. Intolerant	5-3	0.909	1	N/A	N/A	1	1	1
	7-3	0.909	1	N/A	N/A	1	1	1
	9-3	0.997	1	N/A	N/A	0.085	1	0.970
	12-3	0.909	1	N/A	N/A	0.804	1	1
	14-3	0.194	<0.001	N/A	N/A	0.823	0.931	0.349
	15-3	0.706	<0.001	N/A	N/A	0.350	0.717	0.873
	7-5	1	1	0.974	N/A	0.996	1	1
	9-5	0.997	1	1	N/A	0.157	1	0.970
	12-5	1	1	0.028	N/A	0.942	1	1
	14-5	0.028	<0.001	0.198	N/A	0.952	0.931	0.349
	15-5	0.172	<0.001	0.921	N/A	0.544	0.717	0.873
	9-7	0.997	1	0.918	N/A	0.057	1	0.970
	12-7	1	1	0.008	1	0.678	1	1
	14-7	0.028	<0.001	0.062	<0.001	0.700	0.931	0.349
	15-7	0.172	<0.001	0.561	<0.001	0.252	0.717	0.873
	12-9	0.997	1	0.042	N/A	0.607	1	0.970
	14-9	0.077	<0.001	0.283	N/A	0.585	0.931	0.826
	15-9	0.391	<0.001	0.976	N/A	0.966	0.717	1
	14-12	0.028	<0.001	0.823	<0.001	1	0.931	0.349
	15-12	0.172	<0.001	0.135	<0.001	0.978	0.717	0.873
	15-14	0.933	0.987	0.650	0.974	0.972	0.999	0.949